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TOWARDS A NOVEL BIO-INSPIRED DRUG DELIVERY SYSTEM: EXOSOMES LOADED WITH POLYPHENOLS AS NATURAL DRUG CARRIERS IN DIABETES TREATMENT

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Abstract

Polyphenols have been used as active ingredients in pharmaceuticals, healthcare goods, and dietary supplements to improve the functional properties of food. They have been widely investigated *as an antidiabetic agent in vitro* and *in vivo studies*. Polyphenols are known to exert antidiabetic properties as a potent inhibitor or as an activator to minimize β-cell dysfunction and insulin resistance in Diabetes Mellitus (DM). Nevertheless, due to some impaired characteristics such as low bioavailability, rapid elimination from the body, high-rate metabolisms, poor absorption due to hydrophobic properties, and less stability, these compounds are prevented from performing their optimal therapeutic activities. Exosomes, nanoparticles made from different types of cells, have a great deal of potential to be one of the promising carriers in drug delivery systems to address these problems (DDS). To date, exosome exhibits particular benefits as a natural drug delivery agent in terms of specificity, stability, and safety that mediate the synergistic effect between polyphenols and diabetes. Exosomes have recently been used as a novel medication delivery method, increasing the effectiveness and efficiency of the drug delivery system. According to certain studies, exosome-nano encapsulating polyphenols could improve their biological effect in terms of bioavailability and bioaccessability. In addition, accumulated evidence illustrates that nanoencapsulation of polyphenols with exosomes enhances metabolic pathways in DM progression. In this review, we explore the effect of polyphenols encapsulated with exosomes concerning their beneficial effect on DM management.

INTRODUCTION

Regarded as a severe disease, diabetes mellitus (DM) is widely known as one of the major diseases in the world that could cause substantial complications and damage to several organs and tissues of the body, including the heart, brain, eyes, kidneys, foot, and nerves [1]. According to the International Diabetes Federation 10th edition 2021, approximately 537 million adults between 20 and 79 were diagnosed with diabetes, and this figure is expected to rise to 783 million by 2045 [2]. An elevated blood glucose level is a sign of diabetes (hyperglycemia). Blood glucose levels are typically measured between 60 to 140 mg/dL, with a value of less than 140 mg/dL being indicative of hyperglycemia [3]. The inability of the body to absorb glucose into the bloodstream as a result of insufficient insulin production by the pancreas is theorized to be the cause of hyperglycemia. Insulin resistance (IR) or cell dysfunction is thought to be the

root cause of the body's inability to produce enough insulin to maintain a normal level of glucose homeostasis [4]. Besides being genetically inclined, experts believe that the lack of physical activity, obesity, and high body fat are the main factors contributing to the development of IR.

Being overweight or obese is linked to DM due to poor glucose levels, high cholesterol intake, and high glucose levels among patients [5]. Fat accumulation in the muscle and abdomen led to fatty acid breakdown products that could interrupt the insulin-signaling pathways, concurrently inhibiting glucose uptake from the bloodstream [4]. Diabetes patients mostly rely on the administration of commercial oral medications besides a nutritious diet and engaging in regular exercise to mitigate diabetes. These drugs are registered under several classes of drugs that specifically used in treating of DM, where most of them enhance insulin release projection, elevated in secretion and sensitivity of insulin, improve β-cell function, and enhance in glucose uptake. However, some of these drugs pose the threat of serious side effects on the organ long term, such as severe hypoglycemia, infections, pancreatitis, lactic acidosis, anemia, increased risk of hepatic complication, and others [6]. Hypoglycemia could disturb hemodynamics and dysrhythmia, leading to a higher number of cardiovascular events and sudden death. Matthew et al. reported that there is a consistently higher risk of cardiovascular events (congestive heart failure, stroke, ischemic heart disease, and peripheral artery disease) associated with second-line antidiabetic medications (ADMs), i.e., sulfonylureas, the basal insulin treatment, compared with other drugs [7]. Due to the serious health issues of oral pharmaceutical drugs, researchers have shifted to bio-engineered products derived from various natural bioactive compounds by applying different techniques to produce an inhibitor/activator to mimic and have similar actions as commercial antidiabetic drugs. However, the major drawbacks of these compounds that compromise their efficacy are low bioavailability, rapid elimination from the body due to the high-rate metabolisms, poor absorption, less stability, and others [8]. Additionally, several crucial aspects like bioaccessability, matric impact, molecular architecture, and metabolizing enzymes must be assessed and investigated to ensure productivity as a "synthetic drug," rendering them to cope with at least half of the commercial drugs with fewer adverse effects. Hence, to overcome these limitations, a new carrier has been proposed for a drug delivery system (DDS) involving nanoparticles to enhance effectiveness in terms of compatibility, innate stability, transmission efficiency, low immunogenicity, and others [9].

Exosomes are small membrane-based vesicles within the cells that function in various biological and pathological processes [10]. Exosomes are naturally secreted and taken up by different cells in the body [11]. The endocytosis process occurs simultaneously with endocytosis. However, naïve exosomes, unmodified or natural exosomes, rely on their inherited nature to passively target and accumulate in some particular organs, such as the liver and spleen, lowering their targeting efficiency in other organs and weakening the therapeutic efficacy of drugs [12]. Uniquely, these naïve exosomes can be bio-engineered and modified to target specific sites for their biological contents to be delivered through modification on their surface markers by specific proteins/ligands or modification of their core. Unlike naïve exosomes, these modifications can improve targeting efficiency, biocompatibility, high specificity, high sensitivity, selectivity, and affinity [13]. Several studies have demonstrated the effectiveness of these nanoparticles in delivering the desired compounds to affected cells or tissues. In this regard, exosomes that transport biological cargos, including protein, lipid, DNA, microRNA (miRNA), RNA, and fatty acids, have the potential to regulate organ communication, including metabolic signaling pathways in diabetes in a target tissue, altering cell viability and modulating inflammatory pancreatic cell function [14]. Thus, this review addresses several encapsulation techniques involving bioactive compounds within exosomes and summarizes recent studies on nano-encapsulated exosome-polyphenols as one of the bio-therapeutic agents in diabetes treatment.

BIOGENESIS AND EXOSOME COMPOSITION

Extracellular vehicles (EVs) are lipid-bound vesicles secreted by all types of cells into the extracellular space. There are 3 subtypes of EVs that differs in terms of size, composition, and functions, i.e., exosomes (30–150 nm), ectosomes (100–1000 nm), and apoptotic bodies (50–5000 nm) [15]. Exosomes originate from multivesicular bodies (MVBs), which interact with the plasma membrane and have a cup-shaped appearance, whereas ectosomes are EVs that directly sprout from the plasma membrane [16]. Exosomal bilayer walls enclose the cargo to prevent the inner biological material from being lost or damaged until it reaches the target site. They were shown to carry cell-specific cargos, including DNA, RNA (mRNA and miRNA), long-coding RNAs (lncRNAs), and circular RNAs (circRNA), lipid, and protein that can be picked up by nearby or distant cells in a specific manner after release. To date, according to the latest version of the exosome content database, Exo Carta (Version 4, http://www.exocarta.org), 9769 proteins, 1116 lipids, 3408 mRNAs, and 2838 miRNAs have been identified in the exosomes with a different source of origin [17]. The biological molecule that was carried by exosomes originated from their parent cells. This allows them to transmit to nearby or far-off cells the biological information associated with the target cell's specialized cellular processes. [18,19]. Various biological contents enclosed by lipid bilayers have their own specifications and function to be explored in deep (Figure 1). Generally, the biogenesis of exosomes arises from the cell's endosomal systems, beginning with the formation of early-sorting endosomes (ESEs), then moving to the late-sorting endosome (LSEs), which eventually matured into MVBs

[20]. Components in the MVBs are either fused with lysosomes for degradation purposes or they can be fused with the plasma membrane via inward budding and releasing into extracellular spaces, which it called exosomes. Endosomal complex systems are required for transport (ESCRT)-dependent and ESCRT-independent pathways [21]. Four complexes are regulated along with the (ESCRT)-dependent pathways, including ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III, which have been identified and come with associated proteins [21].

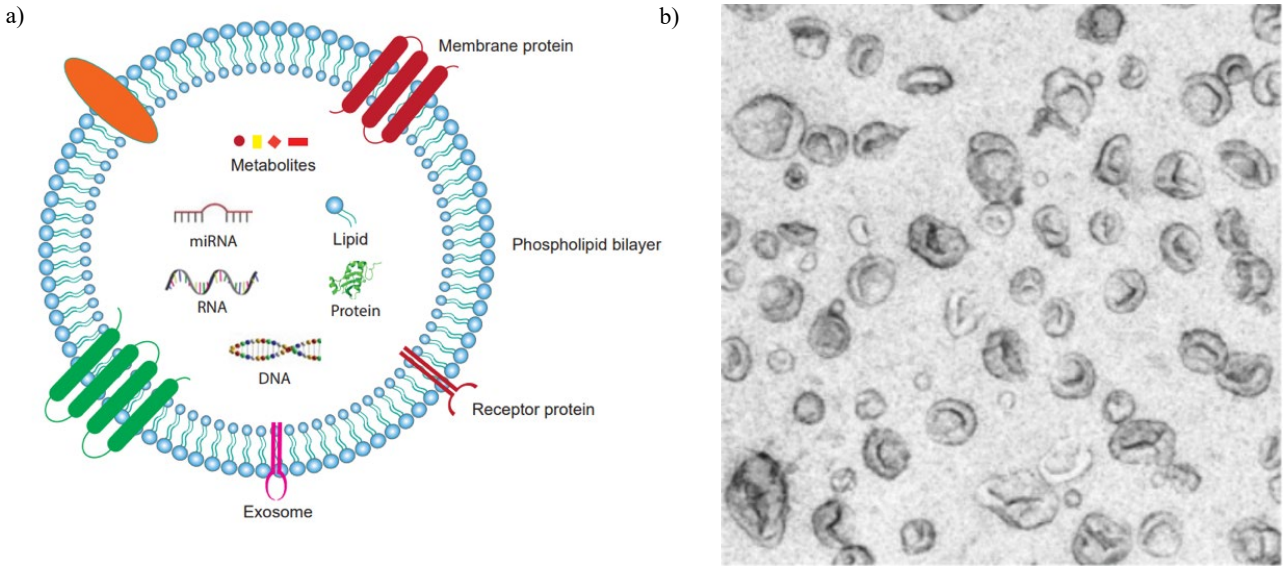

Figure 1. a) Structural design of exosomes containing biological cargo such as nucleic acids, proteins, and lipids. Each component carries out its function to be applied in pharmacological treatments, and b) the morphology of exosomes observed using a Transmission Electron Microscope (TEM) down below 100 nm [18]

Under ESCRT-independent pathways, the ESCRT-0 complex begins by recruiting other protein complexes, such as ESCRT-I, ESCRT-II, and ESCRT-III, to the endosomal membrane. ESCRT-I and ESCRT-II are responsible for the inward budding of the membrane. The membrane invagination and formation of intraluminal vehicles are facilitated by ESCRT-III [22]. The involvement of the ESCRT complexes is proved by the presence of ESCRT components (TSG101 and ALIX) in most of the cell-derived exosomes, which can be one of the exosomal positive markers. Meanwhile, ESCRT-independent pathways are enriched with the accumulation of ceramides, sphingomyelins, cholesterols, and tetraspanins-associated dynamic membranes to release the EVs [23]. Exosomes are taken up by the receiving cells via three main mechanisms, endocytosis/phagocytosis, membrane fusion, and receptor-ligand interaction. Among the interactions, endocytosis is considered the most common and quickest way of exosome uptake. The exosome can then either release its contents to carry out its biological tasks or be broken down by lysosomes for recycling. Exosomes derived from different cells carry their specific protein markers either on the surface or in the cytosol of the exosomes to be recognized by their specific ligands/receptors. However, some common protein markers differentiate exosomes and ectosomes (microvesicles) because, in some conditions, both vesicles are redundant in size. Hence, it is vital to prove the presence of exosomal positive makers that distinguish them from the "contaminants," mainly from the cellular compartments, such as EVs like ectosome, apoptotic bodies, or others. The expression level on these markers also depends on the method used to isolate the exosome and its purity level. Hence, a proper isolation technique and several molecular and analytical analyses must be done to understand the association between exosomes with protein markers. Noticeably, not all surface markers are present, and this also depends on the source of the cell, cell type, and external factors (growth factors, technique of isolation in a time-dependent manner, harvesting technique, and others). According to the literature, several exosome protein surface biomarkers have been positively claimed that are implicated during exosome biogenesis, including transmembrane protein (CD9, CD63, CD81, and CD82), MVBs-associated proteins (ALIX, TSG101, Flotillin-1, and Clathrin), and heat shock proteins (HSP90, HSP60, and HSP70) [19].

POLYPHENOL

The action of polyphenols is largely studied in vitro and in vivo, proposing them as one of the biotherapeutic agents either in small- or large-scale studies. Polyphenols are classified as secondary metabolites synthesized from L-

phenylalanine or L-tyrosine through the phenylpropanoid pathway [24]. Polyphenols derived from natural sources have been associated with health benefits due to their antioxidant properties and many hydroxyl groups capable of scavenging free radicals and chelating metal ions [25]. Most polyphenols have strong antioxidant properties that reduce oxidative stress and are associated with reactive oxygen species (ROS). ROS can be generated through exogenous sources (UV light irradiation, metal-catalyzed reactions, toxins, synthetic solvents, drugs, etc) and exogenous sources (mitochondrial reaction, peroxisomes, inflammatory cell activation [26]. Oxidative stress happens when there is an imbalance production of ROS due to excessive generation of ROS inside the cell or tissue that antioxidant systems in the body are unable to neutralize it in order to maintain cell homeostasis and functions such as gene expression, signal transduction, and receptor activation [27]. Failure to maintain oxidative stress at a normal level can alter the composition of protein, lipids, and nucleic acids in the body can induce mutagenesis of bases, breaking of lipid chain, amino acids modified, crosslink of protein-DNA, breakage of the peptide chain, increased proteolytic and some others effects to occur [28]. Meanwhile, excessive ROS production may cause irreversible damage to cells, undergo an apoptosis state, and eventually lead to cell death. Under normal circumstances, dying cells are recycled by the immune systems, however, in dire consequence, failure in cell clearance can be linked to the major health problem such as cancer, autoimmune conditions such as lupus or type 1 diabetes, neurodegeneration, viral infection, heart attack, and others [29]. Polyphenols can defend against oxidative damage in several ways in various mechanisms. Before cell viability is substantially harmed, polyphenols such as phenolic and flavonoid compounds can interact with ROS to terminate the chain reaction. Polyphenols may act as an efficient free radical ROS scavenger, inhibiting oxidases that are responsible produce superoxide anion and chelating trace materials [30]. Antioxidant activity by polyphenols is considered one of the therapeutic ways for the polyphenols to compute with the scavenging of ROS. The level of antioxidant activity in each of the different polyphenols is varied owning to their respective aromatic structural features, several hydroxyl groups, and highly conjugated systems [31]. Based on the mechanism of flavonoid's potential in scavenging ROS, which is reviewed by Slika et al stated that flavonoids reduce ROS by donating hydrogen atoms and electron to reduce the free radicals, inhibits translocation of nuclear factor, NF-κB in preventing expression of cyclooxygenase (COX-2) that responsible to produce free radicals, and chelate trace materials and thus preventing it from generating free radicals [32].

Current Studies on the Effect of Polyphenols in Diabetes

In diabetes, polyphenols could highly improve glucose tolerance and antihyperglycemic activity. Menchetta et al. reported that oral administration of polyphenol fraction (50 mg/kg) of *Coriandrum sativum* seeds (PCS) and 2 mg/kg of glibenclamide significantly decreased blood glucose concentrations of diabetic mice after 30 mins, attributing to the excellent antihyperglycemic activity [33]. Interestingly, PCS managed to lower blood glucose tolerance compared to the normal control, which manifested high glucose levels. PCS is mainly comprised of nine molecules belonging to the polyphenols. Moreover, the link between the bioactive compounds and DM can be seen as one of the potential activators to stimulate GLUT4 translocation, increasing glucose uptake. Certain polyphenols improve insulin-signaling pathways, which increases insulin sensitivity and secretion, besides acting as a synthetic inhibitor to reduce insulin resistance through the P13K/AKT pathway activation [34, 35]. Besides, polyphenols also preserve functional β-cell mass by decreasing β-cell proliferation, reducing apoptosis, improving insulin secretion, and promoting glycemic control in type 2 diabetic patients [36]. Studies have demonstrated that some polyphenols promote similar results in lowering blood glucose levels and improving glucose tolerance in managing diabetes compared to oral drugs. Yilmazer-Musa et al. studied the inhibition of grape seed and tea extracts on the enzymatic activity of α-amylase and α-glucosidase. The findings revealed that grape seeds demonstrated a much higher potency than the drug Acarbose. Likewise, catechins are potent inhibitors of α-glucosidase activity [37]. Meanwhile, *Primula denticulate* extract exhibited antidiabetic activity through the isolation of triterpenoid saponin (TTS) compound, which can reduce blood glucose by improving insulin secretion from pancreatic β-cells, while the phenolic extract of *Citrus limon* (L.) completely inhibited (100%) angiotensin-converting enzyme (ACE) activity and inhibited α-glucosidase activity [38,39]. Table 1 compiles different polyphenols and mechanisms regulated in DM.

Encapsulation of Polyphenols

Encapsulation is a process where bioactive compounds (polyphenols, micronutrients, enzymes, and antioxidants) are encapsulated by other materials to prevent biodegradation and increase bioavailability to rise the performance of the newly capsulated material [47]. Specifically, encapsulating substance is referred to as the solid shell (polyphenol), meanwhile, the encapsulated substance is referred to as the core substance (exosome). Likewise, nanoencapsulation is applied to overcome the drawbacks of polyphenols, especially low bioavailability and bioaccessability that limit their therapeutic effects [48].

Isolation Methods

Each method uses an exosome characteristic—such as density, shape, size, or surface proteins—to facilitate exosome isolation. The classification of encapsulation

Table 1. Prospective studies evaluating polyphenols action regulated in diabetes mellitus

Samples/ Polyphenol	Cellular mechanism	Physiologic key action	References
Acorn kernels/ Quercetin, azelaic acid, gallic acid	Increase inhibition of α-Amylase, α-Glucosidase, and DPP (IV)	A low blood glucose level	40
Hawthorn/ Epicatechin Procyanidin B2 Chlorogenic acid	Activates SIRT1/AMPK/NF-κB signaling pathway Reduced insulin resistance	Inc. glucose uptake Ameliorate hyperglycemic, inflammatory, and insulin resistance	41
Blue honeysuckle Quercetin, kaempferol, isorhamnetin, Anthocyanins	Inhibit lipid accumulation in adipocytes Suppressed lipogenesis via AMPK activation	Inc. lipid metabolism Inc, expression of beige adipocyte markers.	42
Cacao	Reduces postprandial plasma glucose Increase insulin secretion Increase secretion of GLP-1	Dec. blood glucose level	43
Smilax china L. polyphenols (SCLP)	Improved glucose tolerance fat accumulation, inflammation, and lipid concentrations of serum and liver	A low blood glucose level	44
Avocado Fatty acids, sterols, triterpenes, phenolic acids, and flavonoids	Increased antioxidant activity Increase inhibition of α-Amylase	A low blood glucose level	45
Cambuci *Gallic acid dev.* *Quercetin dev.* *Coumaric acid dev.*	Improved glucose homeostatic Decreased body weight Decreased insulin resistance	Inc. glucose uptake Ameliorate hyperglycemic, inflammatory, and insulin resistance	46

DPP (IV); Dipeptidyl-peptidase 4, SIRTI; Sirtuin 1; AMPK; AMP-activated protein kinase; NF-κB; nuclear factor kappa B, GLP-1; glucagon like peptide 1.

depends on the size of the core material and several methods of loading polyphenols/drugs into the exosome have been invested including incubation, transfection, physical treatments, and in situ assembly and synthesis [49]. Transfection and incubation are frequently employed to deliver the cargo into donor cells. Incubation is proposed as the simplest technique, which involves placing the specific cargo into exosomes and incubating them at a regulated temperature and time with the desired concentration gradient [50]. Although this method seems the easiest and most direct loading method, however, this method has its own drawback since it gives a lower encapsulation and loading efficiency. Normally this method is preferable in encapsulating hydrophobic compounds since these compounds can easily diffuse into lipid bilayer of exosome without needed external force applied. Incubation method also being applied in endocytosis condition where the compounds will be naturally introduced into the exosomes by incubated with host cell starting from the beginning of isolation's process. However, some limitation has occurred since most of host cell cannot be directly incubated with high concentration of doses since it can give effect on the toxicity of the compounds towards the cell and hence reduce the formation pelleted of exosomes. In this case, the toxicity is not only depending on the compounds itself but there is some additional circumference that need to be reconsider in this case such as handling with solubilizing solvent to dilute the compound since most of it are using inorganic solvents to dissolve it. Meanwhile, in the transfection method, specific plasmids are transduced into cells using transfection reagents to ectopically produce desired nucleic acids, proteins, or peptides, which are then packaged into exosomes [51]. Physical treatment involves sonication, electroporation, extrusion, freeze-thaw, surfactant treatment, and dialysis for the encapsulation to take place. Among others, sonication is considered as the most popular and efficient method since it yields higher loading efficiency. To gain this, exosomal membranes are disrupted by sonication and electroporation, that allowing the entry of compounds into the exosomes. Eventually this method is more preferable to be use in encapsulating hydrophilic compounds since some external force is needed for the compounds to pass through exosome's lipid bilayer. However, longer or continuous sonication can cause major drawbacks to the outer layer of exosomal membrane that will break the membrane and this will eventually give effect in downstream analysis since most of the exosomal cargo is being degraded at the beginning of isolation process take place. In electroporation,

an electrical field disrupts the phospholipid bilayer of the vehicles (EVs, exosomes, or cells), allowing the compound to flow through the membrane into the vesicles [52]. Through extrusion method, exosomes are combined with the drug and placed in a syringe-based lipid extruder, where they have extruded through the membranes with porosity sizes of 100 to 400 nm at a regulated temperature [53]. The Exosomal membrane is ruptured and forcefully mixed with the drug during extrusion, resulting in drug loading. In the freeze-thaw method, the drugs are incubated with exosomes at room temperature, frozen at 80°C or in liquid nitrogen, and thawed at room temperature. This step is performed at least three times to ensure encapsulation [54]. Lastly, in situ synthesis is a non-invasive method of loading nanomaterials onto the surface of exosomes or within exosomes [55]. Table 2 shows the different methods applied to cargo-load exosomes that were varied in their encapsulation method in various clinical applications.

Table 2. A summary of various methods for obtaining cargo-load exosomes along with their outcome, as well as their specific applications

Sources	Types of cargo	Method	Outcomes	Applications	References
Black bean (*Phaseolus vulgaris L.*)	Quercetin, β-sitosterol capsaicin	Incubation at room temperature (RT) Electroporation	Electroporation achieves high loading efficiency, enhances the anti-proliferative activity	Cancer treatment	56
HEK293	Curcumin	Transfection	Increased the delivery efficiency of curcumin Exosome membrane-bound RBP had an anti-inflammatory effect *in vitro*. Reactive oxygen species levels were increased	Inflammatory lung cells	57
Stem cell-derived exosomes	Chitosan hydrogel	Incubation	Significantly enriched in terms including neutrophil chemotaxis, chemokine-mediated signaling pathway, inflammatory response, and immune response	Periodontitis	58
Human endometrial stem cells (hints-EXOs)	Atorvastatin	Incubation	Anti-proliferative impact Enhance efficient activity to control tumor	Cancer treatment	59
RAW 264.7 macrophages	PTX and doxorubicin (D OX)	Incubation at room temperature (RT) Electroporation Sonication	Sonication shows the highest amount of PTX-loaded exosome followed by electroporation and incubation at RT Potent anticancer effect	Cancer treatment	60
Adipose mesenchymal stem cells (ASCs)-derived exosomes	Quercetin Vitamin A	Incubation	Enhances the therapeutic efficacy of exosomes improved the liver targeting of exosomes Reduce rapid senescence-like response induced by acute liver injury	Acute liver injury	61
Macrophage derived exosome	Berberine	Incubation	Elevate the M2 protein marker CD206 and reduce inflammatory and apoptotic cytokines good anti-inflammatory and anti-apoptotic effect	Spinal cord injury (SCI)	62

Newly added Factors that can Enhance Encapsulation of Polyphenols

Nanoencapsulation formulations have been proposed to improve polyphenols' stability in drug delivery systems. Nonetheless, the different classes of non-ionic surfactants and their combinations with the nanoencapsulation technique had been widely reported in the literature to have great potential in the activation of the enveloped virus, immobilize, and protection towards protein and enzymes, along regulating the diffusion transmembrane proteins [63,64]. Generally, Tween 20, 40, 80 are a few suitable non-ionic surfactants that have been used because of their high compatibility, non-irritating nature, and stable characteristics. Surfactant is well known for providing a large interfacial surface area to improve drug permeability;

hence it is the most crucial in identifying concentrations of surfactants for the formulations which resulted in improving drug stability [65].

Polyethylene Sorbitan Monoesters (Tween 80 and Tween 20)

A new approach that allow encapsulation to take place with high encapsulation efficiency is moving towards novel applications of surfactants to make up for the shortcomings of nanovesicles. Polyoxyethylene sorbitan monoesters (Tween) is a non-ionic surfactant which had been used as a solubilizing agent of membrane proteins. Garg et al., prepared solid self-nanoemulsifying drug delivery systems (SNEDDS) of polypetide-k, curcumin, Tween-80 (surfactants), Transductal P (cosurfactant), and Aerosil-200 for high therapeutic effects in STZ-induced diabetic rats [66]. Interestingly, this formulation displayed a five-fold rise in the dissolution and permeation rate of drugs, and high stability in a variation of pH, temperature, and dilutions. Dharmawan et al. study on effects of Tween 80 metformin encapsulation on the chitosan-alginate matrix in drug-delivery applications. The addition of Tween-80 was expected to reduce surface tension between the drug, and therefore the drug can be easily infused inside the lipid barrier of exosomes [67]. Comparatively, the inclusion of Tween 80 increased the rate of encapsulation since addition of surfactants reduce particle aggregation since it covers most of the surface of nanoparticles. Therefore, this application can be introduced along with sonication method, where these surfactant increase the rate for drugs permeability without causing damage towards the physical properties of exosomes, hence reduce the rate of recovery since surfactant is normally being commercialized as a food-grade that considered as safe and there is no toxicity of using it. Thus by applying surfactant, this is smartest approaches by protecting exosome's physical stability and thus preventing the degradation of the entrapped bioactive during isolating. Therefore, surfactants offer a better option that coupled with incubation method with giving high bioactive compounds loading with minimal applied of surfactant concentration. As an additional information, normally surfactant is only being used with the lowest amount, which is less that 0.1% to perform its duty to enhance encapsulation process to take place.

Ebrahimian et al. have studied thymoquinone (Tq), bioactive compounds of black seed (*Nigella sativa*) loaded in exosomes by a novel technique with a combination of three methods including incubation, freeze-thawing, and surfactant treatment [68]. The loading of Tq in exosomes with the addition of 0.1% tween-20 significantly enhanced the encapsulation efficiency by 60%. In comparison with Salarpour et al. which loaded the paclitaxel in exosomes via incubation, and sonication procedures only achieved 18.5 and 23% of encapsulation efficiency respectively [69]. Addition of surfactants considerably improved the loading efficiency into exosomes compared with the simple incubation method. The solubilization of Tween-20 in the drug delivery system is crucial, but the mechanistic details remain elusive. Hence, Dresser et al. explored the interactions of Tween-20 and submicrometer-sized vehicles by biosensing approaches [70]. The remarkable outcomes from their study, they found upon addition of a low concentration of Tween-20 below critical micellar concentration (CMC), had produced substantial conformational changes and suggested Tween-20 monomers play an effective role in vesicle swelling and permeabilization which is typically achieved on membrane solubilization before cell lysis. This new approach is eventually proposed a better quality of exosome to be collected for the other uses.

EXOSOMES- NATURAL DRUG DELIVERY SYSTEM (DDS)

The exploitation of exosomes in DDS for therapeutic purposes is due to its advantages, capacity to mediate intercellular communication, and capability to encapsulate various biological molecules like proteins and nucleic acids within the lipid bilayer membrane or in the lumen [71]. Drug delivery refers to using vehicles or carriers to transport drugs or therapeutic agents throughout the body and release them after reaching a particular site. Hence, several sources of drug carriers have been established either in the natural form or synthetic drug delivery, such as polymers, lipid-based particles (liposomes, solid-based particles like micelles and liposomes, polymeric nanoparticles (chitosan), inorganic molecules (carbon nanotubes), metal-based nanoparticles, and others [72]. The DDS offers several advantages as it can increase the accumulation of encapsulated products at a target site, deliver the drugs at a controlled rate, prevent rapid degradation of the drug, reduce the absorption rate, and others [73,74,75]. Nevertheless, the use of synthetic drug carriers is not without some shortcomings, e.g., toxicity from the materials, high production cost, poor stability, and rapid clearance by the reticuloendothelial phagocytic capacity (REPC) and mononuclear phagocyte systems (MPS) [73].

The development of exosomes as a carrier in a DDS continuously evolves with the introduction of new strategies and new findings. Exosomes are claimed to be natural vehicles in DDS, which has significant advantages over artificial vehicles. [76]. Exosomes are excellent delivery vehicles due to a number of factors, including their ability to be genetically modified to express various surface receptors, ability to encapsulate endogenous bioactive molecules with therapeutic effects, target specificity to increase the uptake, can be genetically modified through various of loading genetic materials like drugs and protein molecules, extended shelf like in body's circulation, and their ability to cross a number of biological barriers [77]. By having low toxicity and immunogenicity render exosomes an ideal natural candidate in the DDS [78]. Exosomes must possess features

recognizable by the specific targeted cells or tissue to function as a potential candidate in DDS. The possibility of side effects from active compounds upon entering the non-targeted organs or tissues is reduced with the presence of the targeted sites. Moreover, exosomes have extremely low immunogenicity compared to other carriers, rendering their low cytotoxicity [79]. Morales et al. reported that insulin loaded in the exosomes derived from three types of cell lines, i.e., hepatocellular carcinoma (HepG2), primary dermal fibroblasts (HDFa), and pancreatic β cells (RIN-m) exerted the highest loading efficiency (50%–60%) [80]. The results demonstrated that the insulin-loaded exosomes reduced the glucose level by promoting and enhancing the metabolism in hyperglycemic conditions. Likewise, Wu et al. highlighted that by using a similar encapsulated cargo, insulin-loaded milk-derived exosomes, significantly reduced blood glucose levels compared to normal insulin treatment in diabetes type 1 mice [81]. Approximately 68% of insulin was released after 8 h of treatments, and no burst release was observed. Oppositely, numerous polymer vesicles and liposomes show burst release of macromolecular drugs in an acidic environment and due to this reason, exosomes are a remedy for drug leakage issues since they shield the cargo from undergo enzymatic destruction.

Meanwhile, insulin-loaded Exos exert a significant hypoglycemic effect compared to normal insulin or insulin with free Exos in oral administration. Therefore, the improved oral bioavailability and in vivo hypoglycemic efficacy of insulin demonstrated the viability of Exos as an effective carrier for peptide/protein drugs. By focusing on the exosome as a potential carrier in DDS, Lv et al. revealed the efficiency of bioengineered adipose stem cell-loaded (hASC-Exo) with miR-21-5p, which promotes wound healing in diabetes [82]. The study examined the utilization of exosomes as drug carriers, which revealed their high biocompatibility, increased exogenous miRNA bioavailability, and promoted the healing of diabetic ulcers. Other in vivo studies demonstrated that the combination of hASC-Exo and miR-21-5p promotes re-epithelialization, collagen remodeling, angiogenesis, and vascular maturation, accelerating diabetic wound healing [83]. Delayed wound healing is a major complication that damages peripheral and micro blood vessels and inhibits the expression of various neurotrophic and vascular factors. Exosomes contain a series of bioactive substances vital in wound healing, particularly in promoting new angiogenesis. Since insufficient angiogenesis has been documented in diabetic hearts, it is significant to test whether nano-encapsulated-derived exosomes could assist in angiogenesis regulation. Zhang et al. demonstrated that exosomes at 100 μg/mL encapsulated in PVA/Alg nano hydrogel under the human umbilical vein endothelial cells (HUVECs) exhibit a significantly higher strengthening of proliferation and migration, indicating that nano-encapsulated exosomes significantly enhanced the angiogenic potential of fast wound healing [84]. The in vivo study showed the remarkably reduced size of wounds, which almost returned to the normal state when treated with exosomes.

APPLICATIONS OF POLYPHENOL-LOADED BY EXOSOME IN MEDICINAL FIELD

The challenge to successfully encapsulate exosomes loaded with polyphenols has gained interest in various medicinal fields. However, certain requirements need to be reexamined in order to achieve a successfully encapsulated since many factors need to be, particularly in terms of level toxicity toward the recipient cell, encapsulation method since high encapsulation efficiency needs to be achieved to successfully delivered the ideal amount of polyphenol towards the targeted site without being harmful or giving danger to tissue or organ that can contribute to other health issues. Treated with high concentration of polyphenol can contribute to a serious health issue due to their immediate toxicity or long-term negative effects. Therefore, it is essential to optimize polyphenol-loaded exosome that is highly functional and biocompatible. Here, the next section will be discussed the most recent and available studies related to the encapsulation process that addresses the issue of the impact of exosome-loaded with a polyphenol in various aspects of medical applications.

Cancer Therapy

Cancer is defined as abnormal cell proliferation that can produce life-threatening malignancies and place a heavy financial burden on both patients and the healthcare system. The application of polyphenols in cancer treatment has been studied widely and extensively by focusing on the mechanism that would make this polyphenol exert its activities in cancer treatment Recently, Salek et al. successfully encapsulated polyphenols (berberine, BRB) with human dendritic cells-isolated exosomes and investigated on how polyphenols exert their properties as an anticancer activity regarding breast cancer was studied. Previous literature unveiled that berberine displays high antioxidant, anti-inflammatory, cholesterol-lowering, antidiabetic, anti-obesity, and antimicrobial [85]. The exosome-loaded BRB produced the inhibition of cell proliferation, but the encapsulated BRB gave a more powerful 2- to a 4-time reduction in cell proliferation (42%) with both concentration and time-dependent manner. They also verified that compared to control/native BRB, where cell migration is roughly 50% achieved during 48 hours of incubation, the exosome-loaded BRB exhibits a reduction in cell migration as compared with native BRB. The inhibition of Exo-loaded BRB is possibly caused due to the upregulation of microRNA (miR)-214 that was inherited from the host donor of exosomes, which is a dendritic cell. Overexpression of miR-214 regulates the function and variety of immune cells including dendritic cells, natural killer cells, and macrophages, by playing a bioactive role in

several biological processes such as cell proliferation, tumorigenesis, inflammation, and immunity [86]. Reduction in cell proliferation by native berberine was supported by previous studies done by Sefidabi et al which allocate that berberine inhibited breast cancer proliferation and inhibit cell viability and high expression of berberine could suppress cancer cell proliferation by inhibiting cell kinase complex cyclin A/CDK1 and cell growth-related with P13K/AKT/ERK pathways [87]. In this study, repeated ultracentrifugation has been performed to recover the encapsulated pellet of EVs loaded with BRB. However, there is no release unbound of polyphenol has been done. The likelihood of unbound polyphenols becoming trapped inside the sediments is therefore high, which could reduce the efficiency of polyphenol entrapment. Besides, it was reported that the expression of transmembrane protein (CD63) is slightly decreased after encapsulation treatment and the concentration of exosome through nano-tracking analysis revealed that particle concentration is slightly reduced after EVs loaded with BRB. Indeed, performing repeated ultracentrifugation (UC) at a very high speed may alter the composition of exosomes or give damage the outer layer of lipid membrane in the exosome. Lobb et al also point out that by having repeated ultracentrifugation process tend to reduce the quality of exosomes and hence reduce exosome yields [87]. It is important to address the reduction in transmembrane protein production because this protein can lyse as a result of the ultracentrifugation process's intense agitation. For maintaining a good quality of exosomes, it is advisable to use a mild condition approach with an average centrifugation speed. However, the authors successfully proved that there is no harm or damage to entering the cargo of exosomes since there is no significant difference in terms of cytoskeletal protein as compared with native exosomes.

Towards anticancer activity, another study by Antanio et al highlighted the effectiveness of milk-derived exosomes loaded with curcumin (Exo-Cur) and resveratrol (Exo-RSV) enhance their beneficial effects towards breast cancer [88]. Exosomes are used as medication delivery since free, non-conjugated polyphenols have a poor bioavailability and virtually ever reach human systemic organs. Interestingly, according to the study, free Cur and RSV do not have anti-proliferative effects in mammalian tissues in vivo, but exo-Cur and exo-RSV had a powerful antiproliferative effect on cancer cells for the first time. This point on the role of exosomes as drug carriers in protecting Cur and RSV from being degraded by a rapid metabolism since non-encapsulated polyphenols are not detected upon administration. In the context of anticancer activity, both encapsulated polyphenols induced apoptosis via mitochondrial pathways in a p53-independent mechanism by activation of both caspase-3 and caspase-9. In light of this, the activation of both caspases is supported by a previous study by Peng et al, which show that curcumin positively inhibits cell proliferation and induces apoptosis through the activation of caspase 3 and 9 [89].

Neuroprotective Effect

Neuroprotection can be referred to as action of preserving the loss in the neuron structure and function. The treatment is mainly focused on the option of defending the central nervous system (CNS) against injuries such as traumatic brain injury, trauma, spinal cord injury, and stroke along with chronic neurodegenerative diseases such as Dementia, Parkinson's, Alzheimer's, and epilepsy [90,91]. The most common mechanism underlying neuroprotective include by having elevated levels of oxidative stress, ion balance, increased blood-brain permeability, mitochondrial dysfunction, excitotoxic, inflammatory changes, aggregation of the protein, RNA and etc [92]. It has been hypothesized that oxidative stress is thought to be a major role in the genesis of neuroprotective disease failure since large amount of oxidative stress is likely linked with the majority of neurodegenerative ailments (as stated before). Polyphenols have apparently been demonstrated to have modulatory effects on a number of neurological illnesses by lowering oxidative stress, which is transmitted through brain neurons. Among various polyphenols, quercetin has been reported for having the potential to have a high anti-oxidant that well correlate to the reduction of oxidative stress and demonstrated a promising level of neuroprotection, since it can induce neuroprotection in the treatment of Alzheimer's disease (AD) [93]. Study of an animal model of AD, Qi et al had demonstrated quercetin-encapsulated exosome (Exo-que), to induce neuroprotection by improving cognitive and functional symptoms in the treatment of AD [93]. By applying a passive method (incubation), the results revealed that purified Exo accounted $2.5x10^9$ concentrations of particle, collected from 1mL of rat's plasma, provide a good encapsulation efficiency at 30%. The authors showed that encapsulated Que improved brain targeting as well as significantly enhanced the bioavailability of Que by comparing it with native Que that was administered via intravenous injection with a similar concentration of Que (12mg/kg). In terms of bioavailability, Exo-Que achieved a higher maximum plasma concentration, which is higher to 7.5-fold as compared with free Que, and extend the shelf life of Que after encapsulation for enhancing its bioavailability and brain targeting delivery. In terms of the toxicity of quercetin, no damage to the other organ was located since the morphology of the main organs including the heart, liver, spleen, lung, and kidney was normal and give no difference as compared with the control (saline-treated group). So, this indicates that while preparing Que-encapsulated by Exo, the integrity of Que-primed exosomes was ensured to be maintained, safe, and had no overall systematic toxicity *in vivo.* Exo-Que contributes to its neuroprotective effects by inhibiting Tau phosphorylation through CDK5-mediated phosphorylation of Tau and reducing the formation of insoluble neurofibrillary tangle (NFT), since NFT gives damage to the neuron by causing the neuron to lose connectivity and lastly apoptosis of neuron is finally

happened, leading to the development of AD disease. Hence, this research suggests the therapeutic potential of Exo-Que to improve neuroprotective effects and functional outcomes in the treatment of AD.

Osteoarthritis (OA)

Osteoarthritis (OA), is known as a degenerative joint disease that eventually happens when the cartilage and other tissue in the joint has been a breakdown or gradually deteriorates and has changed in the structure [94]. OA is the most common disease that caused disability in aged individuals. Concerning this disease, curcumin (an active compound derived from turmeric) has been reported to possess inflammatory and anti-osteoarthritic properties, making it a possible therapy for the treatment of OA. To modulate the effectiveness of curcumin in AO, Li et al studied the effect of nano-encapsulated curcumin-primed human bone marrow-derived mesenchymal stromal cells, BMSC-derived Exosomes (Cur-Exo), tends to increase its bioavailability limitation in terms of low stability, rapid systemic clearance, and limited solubility due to its hydrophobic nature to knock down the release of pro-inflammatory cytokines (IL-1β), where these active cytokines released becoming the major risk towards the development of OA, cell proliferation, inflammation, and apoptosis [95]. In this study, a diverse number of assays have been performed such as Cur-Exo labeling and uptake, cell viability including live/dead staining assay, migration (wound healing) assay, and western blot analyte in signaling pathways related to AO. Unlike others, instead of using the exocytosis method in collecting Exo's fraction, this researcher has tested on endocytosis method, where the parent's cell was stimulated before collecting it. This approach is considered the safest and was the most natural way to encapsulated the compounds. However, based on the cell viability data, it shows that the viability of the cell is gradually decreased in a concentration-dependent manner and thus lowering the percentage of Exo-cur collected. By looking at the data, it happened that 24h is the longest time for the cell to be alive and gradually decreased after that. Therefore, due to the limitation of time, there could be many cycles that need to be done to pool enough of Cur-Exo for the downstream analysis. From the findings, Cur-Exo is significantly do not have a significant difference in terms of size, count, shape, internalization rate, viability, or inhibited apoptosis compared with a control Exo's but Cur-Exos is well performed since it upregulated expression of anabolic genes (BCL2, ACAN, SOX9, COL2A1) and downregulated expression of catabolic genes (IL-1beta, IL6, MMP13, COL10A1) more strongly than control EVs, by having high cell viability and reduced apoptosis of IL-1β-treated OA-CH. This study highlighted the potential of Exo's as a natural carrier for curcumin to leash anti-inflammatory effect in AO's treatments.

Diabetes Mellitus

Wound healing is a physiologic, complex condition has occurred when the skin lost the ability to recover and skin function is impaired. Hyperglycemia in DM can cause blood vessel dysfunction, becoming stiffer as well as reduce tissue oxygenation for the wound healing to recover. The risk of wounds in diabetic patients was not only focusing on the fight against infection itself but also rises with long terms persistence along with lowering the immune system's barrier, which leads to life-threatening complications. Referring to this problem, the diabetic wound is hard to recover since this disease is also accompanied by some other factors such as chronic inflammation, immune dysfunction, impaired angiogenesis, and bacterial production, which worsen the healing rate [96]. Recently, Xu et al. reported that polyphenol-modified chitosan hybrid hydrogel (CMGFC) showed significant potential, and improved diabetic wound healing by demonstrating high antibacterial efficiency (>99%) and having a strong antioxidant reactivity (80%) that protected the cell from external oxidative stress, which became an internal factor to reduce the recovery rate of wound healing [97]. Besides, this study also claimed that CMGFC hydrogel reduces inflammatory response by downregulated interleukin-6 (IL-6) and upregulated interleukin-10 (IL-10) levels), promotes angiogenesis, and enhanced tissue regeneration and enhanced collagen deposition in the wound healing process. In response to the potential of CMGFC in wound healing, Zhang et al have created nano hydrogel (exo@H) by encapsulating it with exosomes derived from human umbilical cord mesenchymal stem cells (HUCMSCs) to accelerate wound healing and wound remodelling [98]. Results from this study have found that exo@H could facilitate the proliferation, promote migration and angiogenesis to increase the healing rate of diabetic wound healing due to the presence of protein expressions such as α-smooth muscle actin (α-SMA), CD31, and scavenger receptor, class B type 1 (SR-B1) which could be the marker for angiogenesis. In agreement with this, Liu et al also confirmed the presence of CD31, as a marker of angiogenesis where Exo@H locate the highest protein as compared with naïve exosome and hydrogel control. The present results confirmed exosomes encapsulated with a nano hydrogel could speed up the process of healing where the presence of protein markers via activation of ERK1/2 signal pathways in diabetic wounds. The action of an Exos loaded with hydrogel in wound healing also being investigated by Liu et al, indicated that Hydrogel@Exos noticeably induced the expression proliferation-related protein Cyclin D1 and Cyclin D3 could accelerate wound healing via regulation of LATS1/YAP/β-Catenin signalling [99]. Dephosphorylation of yes-associated protein 1(YAP), involves in Hippo-YAP signalling pathways, translocate YAP into the nucleus and interacts with TEA domain family members (TEAD) transcription factors to promote cell cycle

and anti-apoptosis gene transcription factors being activated, which promote apoptosis in wound healing.

Interpretation of the Effect of Polyphenol-Loaded by Exosome in Insulin Signaling Network-Insulin Resistance (IR) in Diabetes

The insulin transduction system is a metabolic process that response with the binding of insulin with its own receptors, Insulin Receptor Substrate (IRS), which is the major pathways that used to promote glucose uptake into fat and muscle cells and decrease liver glucose production, while contributing to the maintenance of glucose homeostasis [100]. Under a normal state, once carbohydrates intake is digested, body's signaling system allows the for pancreas for insulins signaling. Upon binding with its receptor, the phosphorylation of IR allows activation in p13k pathways to translocate the glucose receptor (GLUT4) into the plasma membrane, which maintains the blood glucose level. However, impair in insulin signaling along with some other disturbance in the system can lead to development of IR. Several mechanisms are associated with IR in the insulin signaling pathways, such as inflammation, mitochondria dysfunction, oxidative stress, physical inactivity, and others [101]. One of the factors that contribute to the development of IR, the release of pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, and other cytokines involved in the insulin signaling pathways [102]. Pro-inflammatory cytokines can disrupt the insulin signaling pathways, stimulating the phosphorylation of insulin receptor substrate-1 (IRS1) and increasing insulin resistance [80]. For example, the production of IL-1β reduces the expression of IRS-1, which lowers the ability of peripheral tissues to use insulin due to IL-1β-inflicted inflammation. This will ultimately be resulting in the development of insulin resistance (IR) in the peripheral tissues. Studies has shown that, another type of pro-inflammatory cytokines, TNF-α can triggers the broad signaling cascade, which activates nuclear factor kappa-B cells (NF-κB) and Jun NH2-terminal kinase (JNK) [103]. Once NF-κB and JNK are activated, they phosphorylate serine 307 in IRS-1, resulting in the impairment of IR-mediated tyrosine phosphorylation of IRS-1. Therefore, the introduction of polyphenols and cargo from the exosomes could help prevent the release of pro-inflammatory cytokines by interception of the insulin-signaling pathway. Figure 2 illustrates the proposed mechanism of polyphenol-exosomes encapsulation involved in the insulin signaling pathways to lower insulin resistance and elevated the uptake of glucose. Insulin resistance can be reduced via three approaches: 1) inhibition of NF-κB since the activation of this pathway regulates the release of pro-inflammatory cytokines, 2) improvement of mitochondrial function through reduction of ROS level, and, 3) enhancement of the translocation of GLUT4 by polyphenols that can mimic insulin to activate certain receptors or substrate in insulin signaling pathways.

Inhibition of Polyphenol Through Activation NF-κB Pathways

miRNA, is a part of biological cargos in the cell-derived exosome that regulates NF-κB signaling pathways by different mechanisms. Some miRNAs play a vital role in NF-κB signaling pathways and NF-κB-associated immune responses and some may act as activators or inhibitors [104]. The activation of NF-κB is stimulated by the presence of diverse stimuli, known as the pathogen-associated molecular pattern (PAMP) and damage-associated molecular pattern (DAMP) from the surroundings [105]. NF-κB activation is initiated when a molecular such as TNF-αbinds to its receptor and triggered enzymatic complex IκB kinase (IKK) leading to phosphorylation of IκB, which resulting IκB ubiquitination and degradation [84]. Once degrade, the remaining NF-κB dimers translocate to the nucleus and activate gene expression of the NF-κB with the release of pro-inflammatory cytokines. Zhang et al reported that Exosomal-miR-146 suppresses the expression of TNF-α and observed a significant reduction of pro-inflammatory cytokine (such as TNF-α, IL-18, and IL-1β) released [106]. Exosomal miR-1249-3p released by natural killer (NK) cells-derived exosomes suppresses the TLR4/ TNF-α /NF-κB signaling pathway but directly targets SKI family transcriptional corepressor 1 (SKOR1), whereby the expression of miRNA-27b by mesenchymal stem cell-derived exosomes reduced the release of pro-inflammatory cytokines by deactivating NF- κB signaling pathways [107, 108].

Polyphenol Improve Mitochondrial Function Through Reduction of ROS Level

Several models have been developed on the effect of ROS in the development inflammation-diabetes related. In the activation process, internal factors such as lysosomal rupture, mitochondrial damage, ionic influx, and protein aggregates induce the excessive level of ROS, leading to the assembly of the inflammasome to take place [109]. The stimulation triggers the activation of pro-caspase-1, leading to the release of pro-inflammatory cytokines (IL-18/IL-1β). The secretion of these cytokines subsequently induces inflammation and insulin resistance associated with proptosis (cell death). Polyphenol is a powerful free radical scavenger that works along with the increase of ROS. Therefore, the balance between antioxidants and ROS could prevent oxidative stress, thus, solving impaired insulin resistance. Consequently, polyphenols have been proven as an indicator to induce the activation of glucose transporter. Dong et al. reported that the action of quercetin had improved tolerance and insulin sensitivity [110]. The dietary quercetin increased the GLUT4 translocation through AMPK activation in 3T3-L1 adipocytes. Most polyphenols are indirect activators of AMPK, targeting the F_1F_0-ATPase/ATP synthase in mitochondria [112]. The activation

of AMPK through the inhibition of mitochondrial function, which also works as a further step in improving insulin sensitivity. Elevated amount of insulin in obesity causes glucose and fatty acids in the blood to promote mitochondrial function, contributing to the pathogenesis of insulin resistance. Hence, the activation of mitochondria also leads to the development of insulin resistance. Treatment of quercetin resulted in the activation of AMPK, which stimulates the oxidation of glucose and fatty acids in the mitochondria. Esebeeri et al. investigated the effects of quercetin on glucose metabolism. The study revealed that quercetin activated AMPK, IRS-1, and AS160 phosphorylation to stimulate the glucose receptor in a basal condition [113].

Polyphenol Enhancement of the Translocation of GLUT4

Likewise, Giacometti et al. also illustrated the same mechanism in the treatment of oleuropein (OL), a phenolic compound extracted from the olive leaf extract, which enhanced glucose uptake and phosphorylation of AMPK and mitogen-activated protein kinase (MAPK) but not the P13K/AKT pathway [114]. Yamashita et al. revealed that polyphenol procyanidins promoted the translocation of glucose transporter by activating AMPK and insulin signaling pathways [115]. Similarly, Nagoya et al. reported black tea polyphenols that promote the translocation of GLUT4 by activating both P13K and AMPK-dependent pathways, inducing the phosphorylation of insulin receptor substrate-1, atypical protein kinase C, Akt Thr308, Akt substrate 160, and AMPK, but did not affect Akt Ser473 [116,117]. Ooi et al. demonstrated that curculigoside and polyphenol-rich ethyl acetate of *Molineria latifolia* rhizome improved glucose uptake via the mTOR/AKT pathways [119]. The study claimed that polyphenol improved glucose uptake with the availability of GLUT4, and the expression is partially mediated through a potential activation of mTOR/AKT pathways. Findings from past studies provide insight into the potential of polyphenols in glucose uptake via varied pathways, with some polyphenols mimicking insulin to perform their action.

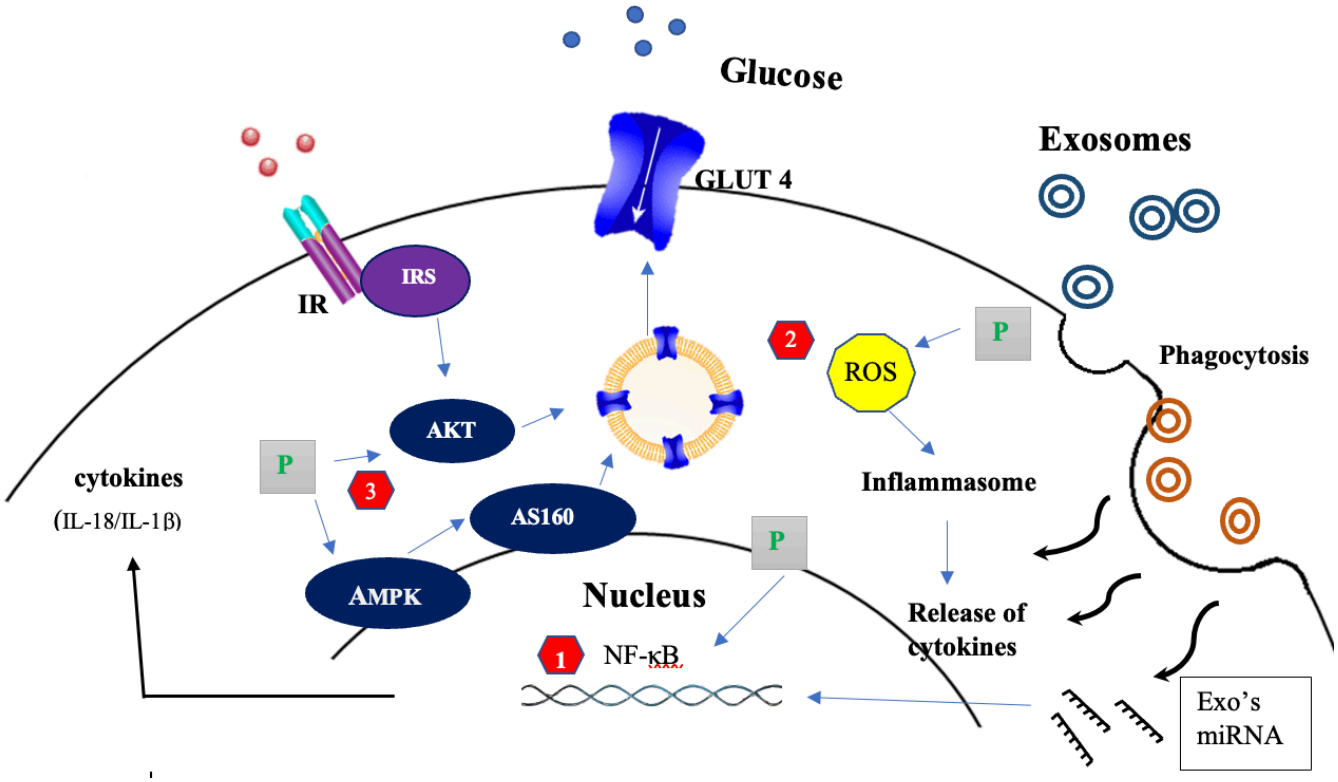

Figure 2. Schematic diagram of the insulin-signaling pathways. The expression of polyphenol-exosomes involved three mechanisms: 1) After reaching the target site, derived exosome-miRNA inhibits the expression of NF-κB-signaling pathways that simultaneously reduced the release of pro-inflammatory cytokines, 2) Derived exosome-polyphenol reducing ROS level through the action by an antioxidant activity carried by polyphenol and thus reduces the released of pro-inflammatory cytokines, 3) Derived exosome-polyphenol released their properties by activation of AMPK, AKT pathways that induce GLUT4 translocation into the plasma membrane, which increase glucose uptake into the cell ;P, polyphenols; ROS, reactive oxygen species; IRS, insulin receptor substrate; Akt, protein kinase B; AS160, Akt substrate of 160 kDa;GLUT4, glucose receptor 4, IR, insulin receptor, AMPK, 5' adenosine monophosphate-activated protein kinase

CONCLUSION

Polyphenol-loaded exosomes offer a promising alternative to diabetes treatment and their improvement as biotherapeutic agents. Exosomes as natural drug carriers offer other benefits besides antidiabetic properties exerted by various polyphenols. Exosomes are well characterized in terms of stability, ability to transport materials to targeted sites in cells or organs, protect materials from degradation, and can translocate between barriers due to the nanoparticle vesicle. However, the mechanism of action for the exosomes-loaded with polyphenols as a biotherapeutic agent in managing diabetes disease is not well understood and characterized, compelling for more investigations to be make since there is a lot of evidence regarding the efficiency of exosome in terms of contribution of internal cargos towards diabetes is being discovered along with effectiveness from variety of polyphenol that comes with different mechanism in treating diabetes. However, to gain a deeper insight into the mechanism of polyphenol-encapsulated exosomes, signaling, behavior, and preference in the body, additional knowledge of their action is indispensable. With this information, it's possible that we'll have a deeper understanding of the signaling behaviors. and preferences.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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