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# AN EMERGING PARADIGM EXOSOMES: COMPOSITION, BIOLOGICAL FUNCTIONS, AND DIAGNOSTIC AND THERAPEUTIC POTENTIALS

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**Abstract:**

*Cells continuously secrete many different types of micro vesicles including macromolecules and micro molecules in to the extracellular fluids. One of them is Exosomes which are nano-sized vesicles capable of transferring the DNAs, microRNAs, non-coding RNAs and lipids with or without direct cell to cell contact, so representing the novel way of intracellular communication. So in this review we tried to summarize the exosome structure, composition, formation, isolation and discuss their active roles in development, function and pathogenesis and their potential use for diagnostic and therapeutic purposes in various diseases. Exosomes are perceived to be used as carriers between different locations of body. Exosomes are almost secreted by nearly all types of the cells and are also found abundantly in the body fluids such as saliva, blood, urine and breast milk. The major role of exosomes is to deliver the information by delivering various effectors or signaling molecules between the specific cells. This review summarizes the current knowledge about the exosomes their function, biological and therapeutic use as well as emerging exosomes based therapies that could not be applied before.*

**Key words:** *Exosome, Microvesicles (MVs), microRNA, Dendritic cells (DCs), drug*

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## INTRODUCTION:

Exosome are the micro level vesicles, and its received lot of attention of scientist over the past few decade (1, 2). Exosomes are the small microvesicles EMV's with the diameter range of 40-100nm and mostly in homogenous shape and can be seen under electron microscope (3). Cells can released different types of membrane vesicles like apoptotic bodies, multiCellular body-derived exosomes and membrane budded microvesicles(1, 4, 5) The word exosome was first derived by Rose johnstone in 1970's because she found this from the sheep reticulocytes that was structurally resembled to the endosome (6, 7). In 1981 Trams et al. exfoliated these vesicles from cell lines with ectoenzyme activity (8). Till the 1990's these exosome considered as best as by-products of cell homeostasis. The revolution in exosome come when β-cells release functional antigen-transforming exosome (9).

**1. Formation of Exosome:**

Exosomes are formed by the endocytic cellular pathway by passing through three different stages: (i) plasma membrane invagination formed the endocytic vesicles; (ii) in second stage inward budding of endosomal membrane is started which give rise the multivesicular bodies (MVB's); (iii) in third and last stage MVB's fused with the plasma membrane and releases the vesicular contents (exosome) (10, 11). So the membrane proteins which undergoes the endosomal pathway exhibits the same stages and exosome are present on their surface. Many different types of lipidic molecules are known for their involvement in exosome formation and release like phosphatidic acid and ceramides (12).

Size of the exosomes are dependent on their site of origin as they are micro vesicles so minimally their sized depend upon lipid bilayer structure in cell (13).

**2. Composition of Exosome:**

Exosomes are unique due to its protein and lipid content which provide the additional hint for their identification. Exosomes mostly contains the fusion proteins and transport proteins (Annexins & flotilin), heat shock proteins (HSP) (HSP70), CD's proteins (CD9, CD81) as well as phospholipases and other lipid related proteins (14, 15). All these proteins can used as positive markers. More than 4400 different proteins are to be identified in association with exosome, by mass spectrophotometer and these proteins serves as cargo for intracellular communication (16). Along with proteins, exosomes are also enriched with lipids like cholesterol, sphingolipids, phosphoglycerides, ceramides and short and long saturated fatty acids chain(10, 15, 17). Research indicates that exosomes serves as to deliver the prostaglandins to target cells (18). Batista et al. in year 2011 investigated that exosomes (Figure: 2) also have saccharide groups in their structure and enriched with mannose and complex an-linked glycan (19). It has been also reported that exosomes also possess the miRNA in a significant amount (13). The cargo function of exosomal RNA is entirely different than the normal cell RNA content (20, 21).

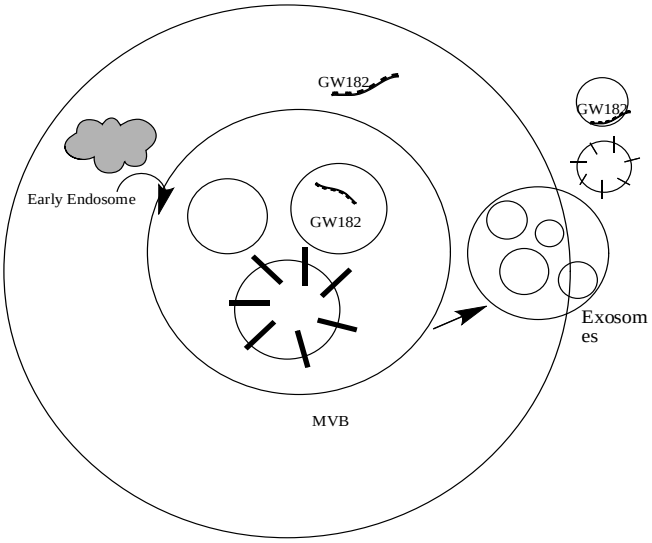


**Fig 1: Schematic Diagram of Exosome production**

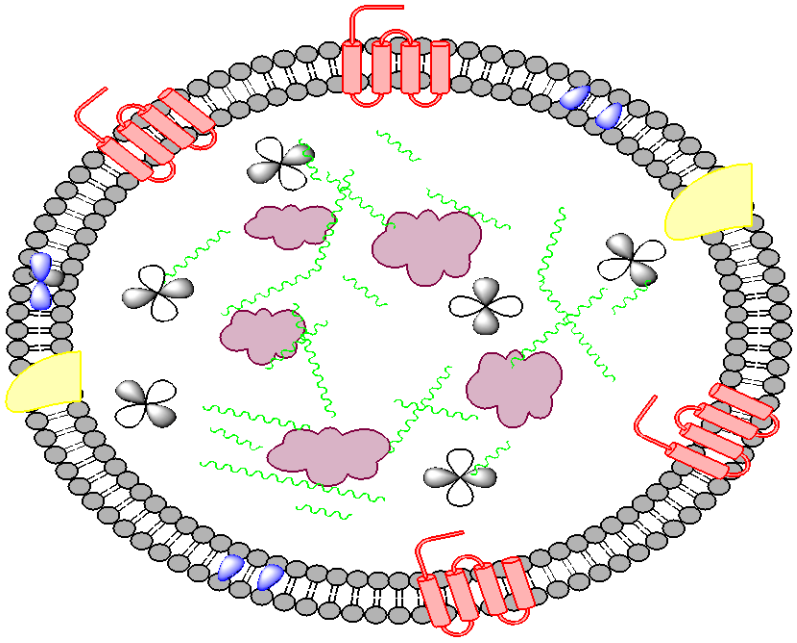

**Fig 2: Representation of mid-size Exosome** (blobs = proteins, green ribbon = RNAs)

**3. Isolation of Exosome:**

Exosome are isolated by the ultracentrifugation method. The centrifugation provides highly rich exosome when is combined with the sucrose cushions/sucrose gradients. Isolation of exosome is based in the size of the exosome (22). Exosome extracted from the blood or cell-culture media is complicated because of a large number of micro-sized particles are present in it, having the same size range as that of exosome (23). Schematic representatation for the isolation of exosome is given below (Figure: 3)

Dilute the plasma/serum (1:1)

⇩

Centrifuge it at 2,000xg, 30min, 4ºC

⇩

Supernatant was collected

⇩

Sediment cells was discarded

⇩

Centrifuge the supernatant at 12,000xg, 45min, 4ºC

⇩

Supernatant

⇩

Stored overnight in a glass tube at 4ºC

⇩

Dilute the plasma with 10ml 30% sucrose cushion

⇩

Centrifuge at 110,000xg, 2hrs, 4ºC

⇩

Sediment = Exosome + contaminating protein contents

⇩

Wash with PBS

⇩

Transfer it into clean ultra tube

⇩

Centrifuge it at 110,000xg, 70min, 4ºC

⇩

Sediment = Exosome

⇩

Shake it with 50-200µl PBS and resuspended

⇩

Store at -80ºC

⇩

Pure exosome

**Fig 3: Isolation of exosome by Ultra-centrifugation method from plasma/serum**

An exciting understanding in the biological significance of exosome was come by the proteomic and transcriptional profiling of exosomes. These studies showed that exosomes were ideal for the biomarker, e.g. CD63, LAMP1 etc (24). In 2007, an noticeable understanding about the exosome biology were carried out, when exosomes were used as natural carrier of nucleic acid taken from the human and mouse (13). However further research on this aspects established that RNA portion of exosomes did not reflects in parent cell, so suggesting the active loading pathways involved loading the RNA with exosomes in the parent cell. The parts of RNA-induced silencing complex (RISC) like GW183 and AGO2 (Figure: 1) which were associated with MVB's and believed that they are involved in miRNA sorting into exosome (13, 25, 26). So the exosomes can give the concept of that it may be use cargoes and biomarkers of different diseases. Exosomes also have diagnostic value and are now being explored as diagnostic tool in infection, cancer and in pregnancy (27).

**Exosomes Biological Functions:**

Now a day's multiple cell lines were described that released the exosomes in vitro like neuronal cells, fibroblast cells, adipocytes, Intestinal epithelial cell and tumor cell lines. In vivo, exosomes are found to be present in many biological fluids like synovial fluid, breast milk, blood, urine and saliva, amniotic liquid and malignant effusions of ascites. In blood serum exosomes are almost present in quantity of 3,000,000 per microliter (7, 28, 29). The first reported biological function of exosomes is as proteins which are expelled out from reticulocytes during the process of maturation in the erythrocytes. Authors believed that particles sediment from the blood plasma at 10,000*G are circular in nature and named them as exosomes (17, 30, 31). Further research showed that, exosomes secretion is just like the excretion process so that get rid the unnecessary protein and RNA (32). But as the day's passed more and more research were conducted on exosomes and this was discovered that exosomes were found to be secreted by many cells types. On the basis of their origin exosomes performed variety of functions. Extensive studies had done on the facilitator effect of exosomes in immune response (33) and its antigen presenting role had been extensively reported (34). Exosomal role in coagulation, inflammation and angiogenesis were also reported (35). After the activation, platelets secretes exosomes as well as other shedding microvesicles (36). In this case exosomes did not perform any role in coagulation but it performs some unknown function. It has been reported that exosomes are involved in Dictyostelium cells migration by using chemo-attractant signals (37). One another group of researchers studied the level of miRNA in exosomes of human breast milk for several months during lactation (38). They were reported that, certain miRNA's like miR-155 and miR-181a that play an important role during immune regulation and were present in high concentration during first six months of lactation were significantly reduced afterward (37). Recent studies demonstrated that exosomes were not only involved in triggering the downstream signaling but they also specifically target the recipient cells and exchange proteins. Exosomes also delivered the specific nucleic acids and worked as cargo (13,39, 40). Exosome most unique function is cell to cell communication especially between the far distance cells in the body. Similarly Exosomes play a unique role in spreading various pathogens like virus and prion form one cell to another (41). Pegtel et.al. (42) showed that miRNAs which was secreted by the Epstein bar virus (EBV) were transferred by exosomes to the uninfected recipient cells.

**Exosomes in Diagnostics:**

From last few years, bundle of research were done on exosomes on their diagnostic aspect. This research discovered that almost all the body fluids contained exosomes (blood, saliva, milk, urine). Because of the unique structure of exosomes, like protein, lipid and RNA, it may be useful for the diagnostic purposes (43). In the late 1970s presence of MVs is derived from the cancer cells in person suffering from hodgkin's disease(44). Since that day till to date considerable efforts have been done to use microvesicles as diagnostic tool (Table: 1). It was reported that MVs levels were elevated in serum, urine and blood in the cancer patient (45, 46). However, microvesicular components may provide the important information regarding disease. For example, for the diagnosis of adenocarcinoma mucin bearing MVs are used as diagnostic marker (47). A proteomic investigation of urine identified the eight proteins which were the important bladder cancer diagnostic tool (48). So that we can say that, protein portion of the exosomes are the useful tool for the diagnosis of the diseases. In addition of this, recent studies showed that cancer patient have different pattern of RNA and mi RNAs. In cancer patient, RNA and miRNA have been found in circulating MVs form (49).

**Table 1: Circulating Exosomes as potential diagnostic markers for various diseases**

| | Sample type | Marker | Disease | References |
|---|---|---|---|---|
| **Quantity** | Plasma | PMPs level | Gastric cancer | (50) |
| | Serum | PMPs level | Prostate cancer | (51) |
| **Protein Expression** | Ascites | CD24, EpCAM | Ovarian cancer | (52) |
| | Serum | Tissue factor | General cancer | (53) |
| | Plasma | Tissue factor | Breast cancer | (47) |
| | Pleural effusion | SNX25, BTG1 | Mesothelioma | (54) |
| | Urine | Fetuin-A | Acute kidney injury | (55) |
| **miRNA or mRNA expression** | Serum | Glioblastoma | Glioblastoma | (56) |
| | Serum | MAGE-1, HER-2 | Gastric Cancer | (45) |

*PCR of the miRNA is sensitive and stable method for the diagnosis and detection of miRNA in patient serum is a new promising approach to detect disease in early stages. Down regulation of miR-92a in plasma is the biomarker of hepatocellular carcinoma and leukemia (57-59).*

**Exosomes as target drug delivery vehicles:**

Exosomes can be used as cargoes so it was believed that it may be used as a targeted drug delivery system. Alvarez et al. first of all presented and proved this hypothesis (60) by using immature dendritic cells. They used the immature dendritic cells (DC) derieved from the bone marrow of mouse as a source of exosomes and these exosomes were devoted as stimulatory molecules such as MHCII and CD80. They purified the exosomes by ultracentrifugation method and used as cargoes for siRNA delivery both in vitro and in-vivo studies. They selected brain as a target tissue in body, because it is believed that blood brain barrier is the major obstacle in the drug delivery to the central nervous system. Sealed functions of BBB is due to the capillary endothelial cells that are tightly sealed by junctions and regulates the barrier functions (61, 62). For the ensurance of targeted exosome delivery, they used the novel strategy by utilizing LAMP2B an exosomal surface protein, that display the targeted peptide on its surface (27).

**Treatment of brain inflammatory disease by encapsulated exosome with drug:**

Zhuang et al, used the encapsulated curcumin (Exo-cur) or JS1124 (Exo-JS1124) inhibitor of signal transducer and activator of transcription and delivered it in to the microglia cells by the intranasal route. They used the lipopolysaccharide induced inflammatory (LPS) model for the experimental mice to induce the inflammation. They showed that, in their study that mice treated with the Exo-cur and Exo-JS1124 were protected from the LPS-induced inflammation. They believed that by the intranasal delivery of exosome was selectively taken by the microglia cells and subsequently induce the apoptosis of the microglia cells (63).

**Exosomes a new approach for treating arthiritis:**

Dendritic cells (DC) and T-cells were used for the delivering of immunosuppressive cytokines in treatment of various collagen induced inflammation in different mouse model(64). DC is the antigen presenting cells and regulates the immune activity. Various factors are involved to stimulate or suppress the immune response of DC, most important is the differentiation. They have the low level of MHC and other molecules like CD40, ICAM-1 so they can suppress the T-cell immune response. The immunosuppressive ability of DCs enhanced its genetic modification and genetically modified DCs showed dramatic control in progression of autoimmune diseases like diabetes and arthritis (65-67). DCs with viral vectors expressing the immunosuppressive agents effect more pronouncedly than T-cells or fibroblasts (68). Due to the ability of genetic modification of DCs, they produced distal therapeutic effects specially when exosomes were delivered along with it locally (69, 70). Immunosuppressive DC exosomes can modify the endogenous immune cells, such as APCs so it may be responsible for anti-inflammatory effects (71).

**Exosomes in Immunotherapy and Nervous system:**

Exosomes/MVs have cell to cell communication function for transfer of genetic material (72-74). The dramatic progress in the research of MVs for drug delivery is due to its low immunogenicity and unique delivering properties. With the help of the genetic engineering EMVs are used to transport the therapeutic drug either by direct insertion or by loading onto the targeted gene (75, 76). Exosomes also serves as excellent therapeutic cargoes due to its protection rendered to enclosed content when packed in mRNA, siRNA, proteins and drugs, and it preserved from degradation. Due to these possible

advantages exosomes/EMVs mediated therapy is actively studied and used in three different main fields, Immunotherapy, RNA-interference (Figure: 4) and drug delivery (77). The most widely investigational portfolio of EMVs/Exosomes is in immunotherapy. Immunotherapy is an ideal technique in cancer treatment. In immunotherapy, vaccines are prepared containing antigen presenting cell to recognizes the tumor cells (78). Rapsoe et.al. showed that B lymphocytes secretes EMVs and these EMVs contained MHC II which can induces the cell response in vitro (9). These exosomes/EMVs may be used as emerging therapy in the treatment of various nervous system diseases. Sun et.al. first developed the drug loaded exosomes (75). They used the curcumin an anti-inflammatory agent and successfully loaded it into EMVs. This exosomal curcumin significantly reduced the LPS-induced inflammation.

**Role of exosomes in Senescence and aging:**

Senescence is the cellular part of aging of tissues due to the irreversible growth arrest and these and other physiological changes occurred in cell morphology, cell behavior and function. miRNAs is the small non-coded RNAs which regulate the gene expression and play an important role in biological processes. Recent development showed that, exosomes contained miRNAs and released into variety of cells and play an important role in cell-to-cell communication and information transfer from one cell to another. Exosomes with miRNAs formed the complex cellular network senescence and contribute to aging (79, 80).

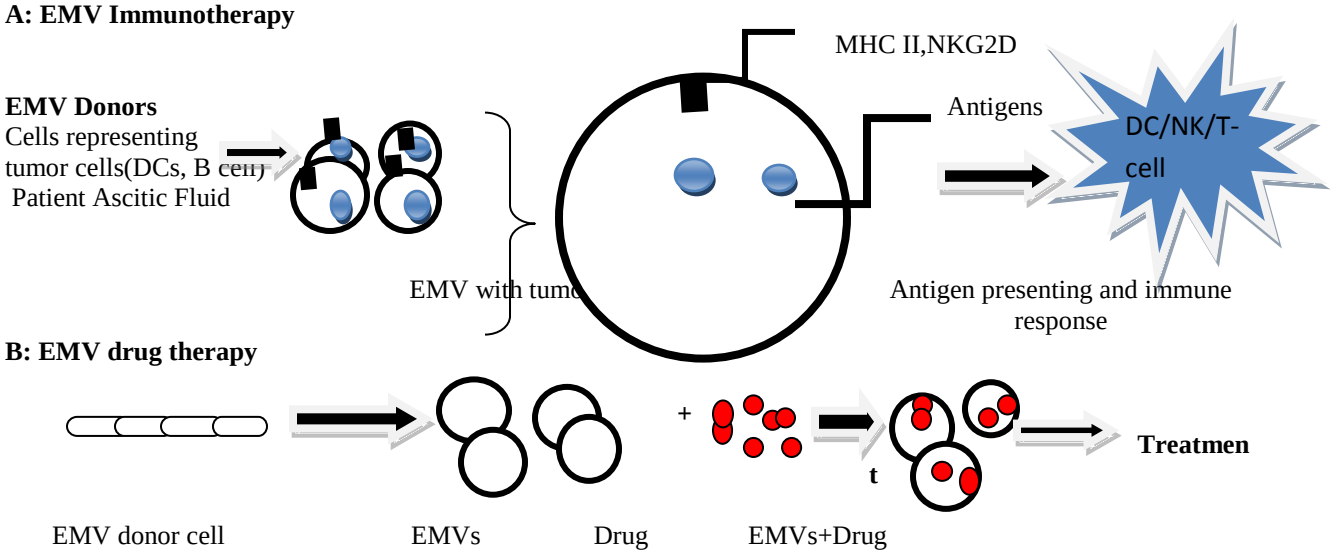


**Fig 4: Extracellular membrane vesicle therapy (EMVs) A: EMV immunotherapy. Tumor antigen on the membrane surface from different sources was introduced *in vivo* to elicit targeted immune responses. B: EMV drug therapy. Drug packaged into/onto EMVs isolated from donor cells to minimize degradation and increase delivery to intended sites.**

**Anti-tumorogenic role and clinical role of tumor-derived exosomes:**

The protein portion of the exosomes reflects the cell type specificity for their cell of origin from which they are secreted. Particularly exosome which are derived from tumor may contain tumor specific antigens on the surface as are present in tumor cells (81). Tumor antigens such as Carcinoembryonic antigen (CEA) (82), mesothelin (83) and silv (84) are observed in tumor-derived exosomes. Due to this observation it is suggested that, tumor-exosome-based cancer vaccines may be developed. Tumor derieved exosomes might be used as a tumor antigen source which might be to able to induce the CD8+T-cell dependent antitumor effects in mice (85). Recently, it was reports that dendritic cells loaded with tumor exosomes elicited the CD8+T-cell response against the tumor cells in malignant gilomas patient (86). For augmenting anti-tumor activity/immunity tumor derived exosomes has been investigated for direct application (Table: 2). Research showed tumor derived exosomes produced the specific antitumor activity when its parent cell were genetically modified. These genetically modified exosome can express pro-inflammatory cytokines such as IL-12, IL-2 etc or which were then was subjected to the stress condition (87-89). Heat shocked lymphoma cells which releases the exosomes expressing MHC and other co-stimulatory molecules and they induce efficient anti-tumor T cell immunity (90).

**Table 2: Studies on the immunogenicity of tumor-derived exosomes and their vaccines**

| Exosome source | Modification | Model | Results | References |
|---|---|---|---|---|
| Mouse colon carcinoma and melonoma | Parent cells were heat treated | Mouse | Elevated level of Hsp-70, elicit Th 1 response | (91) |
| Mouse lung carcinoma | Parent cells were heat treated | Mouse | Activates DCs and T-cells and produce immune response | (92) |
| Human renal cancer | Parent cells were modified to release GPI-IL-12 | In vitro | IL-12 permotes the release of IFN-α | (88) |
| Ascites from colorectal cancer | Exosomes were purified | Phase 1 clinical trials | GM-CSF induce beneficial tumor specific CTL response | (93) |

As discussed in table 2 tumor-derived exosomes are appear to be safe in different clinical approaches but they lack clinical efficacy as were also observed in many animal tumor models. It had been studied because exosomes have potential immunosuppressive agents but those exosomes which are derived from tumor cell upon direct application may promote tumor growth. So the clinical studies were focused on tumor derived exosomes usage which were loaded with DCs (94) or ascites derived exosomes (93). But still many clinical trials on the patients did not evaluate the efficacy and promising effects. It has been observed that circulating exosomes level and tumor marker exosomes increased in cancer patient than the non-malignant patient and increases the tumor progression (95, 96) and furthermore exosomes were isolated from the serum of patient with oral cancer or ovarian cancer (97, 98). Therefore, it was considered that cancer patient may have improved antitumor immune response after removing of immunosuppressive tumor derived exosome form patient blood. In this way progression of cancer might be delayed. San Diego biotechnology company Aethlon medical was developed using novel hollow-fiber cartridge system which can selectively depleted the virus by using the lectin based resin due to its high affinity of glycosylated viral proteins (99). By using this system HIV particles removal has been demonstrated. This system gives the attractive device by which depletion of exosomes whose sizes are similar to the viral particles were occurred (100-102).

**Other uses of Exosomes:**

In 2000s, large numbers of Phase 1 studies were completed on exosomes. The first study used the vaccination against the metastatic melanoma disease with autologous dendritic cells (DC) derived exosomes (DEX). DEX contained functional MHC/peptide complexes that were capable of generating the immune responses. These researchers also established the good manufacturing practice for the manufacturing of pharmaceutical grade exosomes on large scale. They used the exosomes in vaccination by direct loading method. In direct loading method, they attached the peptides directly to the exosomes in an acidic media (103). A similar Phase 1 study of DEX immunotherapy was used in patients with small cell lung cancer (104). In another Phase 1 study exosome which are derived form autologous ascites derived exosomes was used along with granulocyte-macrophage colony stimulating factor (GM-CSF) for the treatment of colorectal cancer (CRC) (93). A phase II trial was recently conducted on patients. After conventional chemotherapy a phase II trial was conducted on patient with non small lung cancer. They used the Dex vaccines in exosomes in which IL-15Ra and NKG2D along with cyclophosphamide agter the administration of chemotherapy based as platinum(105, 106). Exosomes was used for the removal of the unwanted and harmful molecules from the cells (30, 107). Exosomes are useful protein without sequences for secretion, but interestingly exosomes can eliminates the drug molecules from the cells, and the drugs eliminated by exosomes would made the cell resistant to those drug eliminated by them. (108, 109). Exosomes have the ability to pump out the various chemotherapeutic agents from the cells.

## CONCLUSION:

From the past decades, studies for the understanding of the biology and functions of exosomes as well as microvesicles have been exponentially increased. The most precise definition of exosome is this it is the nanoparticles released from the living cells which have around 1.12-1.20g/ml density in sucrose solution. As the days passed more and more research are done on the exosomes from that we are gaining

knowledge on mechanism of formation, secretions, in vivo pathways, and biological role of their nucleic acid, protein and lipid cargoes of exosome. With the emergence of exosomes/EMVs responsible for cell to cell communication, researchers gathered the information on its role both on physiological and pathological functions as well as their use in different therapies. The most interesting aspect about exosomes remain the same of their using as vesicular carriers and serves as cargo. They carry the large sized molecules such as RNA and proteins that influences the gene expression. These microvesicles are similar to viruses and capable of communication from one cell to another cell, can easily pass the contents of cell across the cell membrane and deliver the macromolecules that are biologically active. Due to different cellular origins and biogenesis of exosomes different isolation procedures were established by the research community. Many researches had been done on the therapeutic application of the exosomes. Further developments are aimed to ensure the therapeutic functions and clinical potential of exosomes, including the cargo property, targeting function and different sources of exosomes that enable the tissue targeted application of exosomes.

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