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1 **Title**

2 **Exosomes: A Novel Therapeutic Paradigm for Treatment of Depression**

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17 **Abstract**

18 Extracellular vesicles (EVs) of endocytic origin are known as exosomes. These vesicles are  
19 released by cells and are accessible in biofluids, such as saliva, urine, and plasma. These  
20 vesicles are made up of small RNA, DNA, proteins and play a vital role in many  
21 physiological processes. In central nervous system (CNS), they participate in various  
22 physiological processes such as stress of nerve cells, communication between the cells,  
23 synaptic plasticity and neurogenesis. The role of exosomes in depression needs to be explored  
24 further. It is known that exosomes can cross blood brain barrier (BBB), which is made up of  
25 glial cells astrocytes. One of the advantages of these vesicles is that they are able to transfer  
26 macromolecules like DNA, protein, mRNAs and miRNAs to recipient cells. This review  
27 focuses on the potential role of exosomes in depression and their utilization as  
28 atreatmentoption or diagnostic tool of depression.

29 .

30 **Key words:** Depression, Exosomes, BBB, Serotonin, Biomarker, Oxidative Stress

# 1        **1. Introduction**

## 2        **Major Depressive Disorder (MDD)**

3        Depression or MDD is the most common mental disorder that affects more than 264 million  
4        people worldwide [1]. It is a leading cause of disability globally. Moreover, depression also  
5        increases the risk of suicide attempts [2]. Women are more susceptible to get depression as  
6        compared to men [3]. There are many psychological and pharmacological treatments  
7        available for depression and most of the treatment approaches work through modulation of  
8        the levels of biogenic amines in the CNS [4]. However, the available therapeutic approaches  
9        are limited in their role to control resistant cases of depression as well as therapeutic efficacy  
10       of medications take more time to subside the symptoms of disease [5]

11       There are various psychological, environmental and biological factors involved in the  
12       pathophysiology of depression [6]. Now genetic link is also found and validated. The genes  
13       related to brain derived neurotrophic factor (BDNF), 5-hydroxy tryptamine transporter (5-  
14       HTT), and Norepinephrine transporter (NET) have been identified [7]. Multiple theories and  
15       hypotheses are coined to explain the pathogenesis of depression in that monoamine theory is  
16       widely acceptable. The monoamine hypothesis relies on the abnormally excessive  
17       biotransformation of major neurotransmitters (5-HT, norepinephrine and dopamine) [8]. The  
18       illustration of MDD pathogenesis involves genetic predisposition, deranged monoamine  
19       synthesis/function, and altered structure/function of brain. In addition to above mentioned  
20       factors, depression is also associated with increased oxidative stress due to the formation of  
21       reactive oxygen species (ROS) and imbalance in oxidant and antioxidant signaling [9]. Brain  
22       is more susceptible to oxidative stress due to more consumption of oxygen, higher lipid  
23       contents and weaker antioxidative defence. Dysregulation of hypothalamic pituitary adrenal  
24       (HPA) axis also associated with pathogenic progression of depression [10]. Moreover, in  
25       depression, the levels of various inflammatory markers are increased in blood and CNS area.  
26       Elevated levels of C-reactive protein and cytokines such as interleukin 6 and tumour necrosis  
27       factor-alpha were observed [11]. Treatment with anti-depressants suppresses the  
28       inflammatory response, whereas electroconvulsive therapy acutely increases the levels of  
29       various proinflammatory cytokines [12].

30       MDD also has strong relationship with cardiovascular disorders and is linked with high death  
31       rates [13]. Some previous reports have demonstrated that depression increases the risk of  
32       developing cardiac disease, specifically coronary artery disease, and exacerbates the

1 prognosis after myocardial infarction [13,14,15]. Anxiety and panic disorders are another  
2 disorders found in comorbidity in approximately 80% of MDD cases. MDD also increases  
3 the risk of drug abuse and alcohol consumption [16,17].

## 4 **2. Extracellular Vesicles (EVs)**

5 Since there is no effective therapy available for the treatment of depression and associated  
6 comorbid disorder, innovative approaches should be investigated. In this review we will  
7 discuss the role of extracellular vesicles, including exosomes as therapeutic target and  
8 biomarker for the treatment and early detection of symptoms of depression. EVs are shed by  
9 most of the cells that release them into the extracellular space. These vesicles have very  
10 specific and important role in signaling and communication between cells. The vesicles  
11 mainly categorized in three sub types 1] apoptotic bodies (500-2000 nm); 2] microvesicles  
12 (50–1000 nm); and 3] exosomes (40–200 nm) [18]. They have different biological properties.  
13 Apoptotic bodies are engulfed by macrophages while microvesicles contain a range of cargo  
14 that is delivered to neighbouring cells. Exosomes are developed from intraluminal vesicles  
15 (ILV) via inward budding process of multivesicular body (MVB). They fuse with plasma  
16 membrane that release its ILV contents as EVs called exosomes into the extracellular space  
17 [19,20]. Exosomes contains proteins, lipids, and nucleic acids. They are highly enriched with  
18 microRNA (miRNA). Disease states leads to altered expression of miRNA in exosomes  
19 making the miRNA cargo intriguing candidates for investigation [21]. EVs are lipophilic in  
20 nature and easily able to cross BBB. This specific property of exosomes makes them a target  
21 of choice for the treatment or early diagnosis of CNS disorders like depression [22,23,24].

22 EVs can be isolated from serum, saliva, cerebrospinal fluid, urine, breast milk, synovial fluid,  
23 effusions, semen and cell cultures [25]. The isolation of EVs is done via centrifugation at  
24 100,000 g and/or mechanical filters, which are specifically designed according to diameter  
25 preferred EV subtype. Limited number of research studies is conducted to evaluate the role of  
26 exosomes in mental disorders.

### 27 **2.1 Blood Brain Barrier (BBB) Penetration and Extracellular Vesicles (EVs)**

28 Effective Delivery of drugs to the brain for the treatment of central nervous system (CNS)  
29 disorders for examples neurodegenerative diseases, tumors, trauma, stroke, autoimmune  
30 diseases is a major challenge [27, 28, 29, 30]. BBB is a border that limits and regulates the  
31 passage of substances between the peripheral vascular circulation and the CNS, thereby

1 providing protection to the CNS from toxic substances or overactive immune responses  
2 [28,31]. However the BBB permits transmembrane diffusion of lipid soluble molecules of  
3 size less than 400 Da and selective transport of some compounds into and out of the brain  
4 [32].

5 Two plausible transport mechanisms across the BBB were proposed namely transcellular  
6 through BMECs, and paracellular through junctions between BMECs. The main components  
7 of BBB are microvascular endothelial cells (BMECs), astrocytes, pericytes, the endothelial  
8 basement membrane, and neighbouring neurons. A complex arrangement of tight junctions  
9 (TJs) and adherens junctions (AJs) in the brain endothelial cells regulates paracellular  
10 permeability [33].

11 . Around 98% of small molecule drugs and almost all of large molecule biologic drugs,  
12 including recombinant proteins, monoclonal antibodies, or gene-based medicines are unable  
13 to cross the BBB [34,35]. Exosomes with its contents are capable of crossing the BBB. This  
14 finding has promoted biomarker research with exosomes and their use as a drug delivery  
15 system. Numerous studies have demonstrated the successful brain delivery of exosomes.  
16 Effective delivery of GAPDH siRNA to the brain via systemic injection of exosomes in mice  
17 [36] was reported. Other experiments have been proved to be successful in delivering  
18 exosomes to the mice brain via intranasal injection [37]. In another experiment using rats, a  
19 fluorescently tagged protein selectively expressed in rat brain tissue was recovered in small  
20 EVs having same characteristics as exosomes in their blood [38]. These studies support the  
21 fact that exosomes are able to communicate from the brain to the rest of the body and they  
22 can cross the BBB in a bi-directional manner. However, their exact mechanism of BBB  
23 penetration remains unclear.

24 In an experiment using in vitro BBB model the transfer of EVs derived from human  
25 erythrocytes was found to be dependent on the adsorptive-mediated transcytosis method of  
26 transport [39]. Though the EVs crossed under healthy and inflammatory conditions, the  
27 peripheral administration of lipopolysaccharides was found to considerably increase the EVs  
28 movement across the BBB [39].

29 Another study revealed that in healthy and stroke-like condition exosomes crossed a BBB  
30 model using transcellular BMEC endocytosis, suggesting that exosomes maintain their ability  
31 to cross during stressful conditions [40]. This same study also indicated that exosomes were  
32 internalized through endocytosis, and accumulate in endosomes. There was a decrease in

1 exosome transcellular migration using chlorpromazine (CPZ) an inhibitor for clathrin-  
2 dependent endocytosis, which transfers clathrin from the surface of cells to intracellular  
3 endosomes [40,41]. This indicates that clathrin-dependent endocytosis may be involved in  
4 transportation of exosomes across the BBB [40].

5 Studies have indicated that methyl- $\beta$ -cyclodextrin (M $\beta$ CD), which removes cholesterol from  
6 the plasma membrane [41], and filipin III, which binds to cholesterol caused a significant  
7 reduction in BBB penetration of exosomes [40]. This result revealed caveolae-dependent  
8 endocytosis as alternative route of exosome migration. Most likely the uptake of exosomes  
9 in BMEC depends on specific ligand receptors or lipid rafts, and mechanisms of exosome  
10 uptake may also depend on the cell for which they originate. It has been postulated that  
11 exosomes from different cell origins have different content including protein and lipids,  
12 possibly modifying their mechanism of BBB penetration [42]. Moreover, disease condition  
13 may influence the mechanism of BBB crossing, as the contents of exosomes change upon  
14 disease state [26, 42].

15 Studies have also proposed that exosomes play a role in increased permeability of vascular  
16 barriers of the BBB. Breast cancer cells secreted exosomes uniquely expressed miR-105,  
17 which directly targeted the tight junction protein ZO-1 [43]. This exosome transfer of miR-  
18 105 destroyed tight junctions and the integrity of the BBB [43]. Moreover, claudin-5 (Cldn5)  
19 a tight junction protein of the BBB was found to be encapsulated in exosomes [44]. A  
20 loosened BBB was found in the Cldn5 knockout mice [45], suggested that exosomes carrying  
21 Cldn5 might play a role in BBB integrity. It was also found that a decline in Cldn5 induced  
22 depressive-like behaviors in mice, and antidepressant treatment increased Cldn5 levels and  
23 promoted recovery [46]. A leaky BBB has been linked to neuroinflammation [47,48]. Thus,  
24 the possible influence of exosomes on the BBB integrity also advocate a role for exosomes in  
25 neuroinflammation and the pathogenesis of mental disorders [47,48] Hence, in mental  
26 disorders a leaky BBB state may be caused by exosomes released from cells being influenced  
27 by this disease state.

## 28 **2.2 Signalling mechanisms involving exosomes in the brain**

29 An important role is played by exosomes in cell to cell communication in the CNS..They are  
30 involved in signalling through distal as well as neighbouring cells [49]. The exosomes work  
31 as an important link for communication with similar kind of cells or also with different types

1 of cells. The release of exosomes in the CNS is controlled by glutaminergic neurons and  
2 influx of Calcium ions [50,51].

3 N-methyl-D-aspartate (NMDA) is a glutaminergic receptor. The entry of calcium ion through  
4 gates of this receptor triggers the neuronal release of exosomes. Other important  
5 glutaminergic receptor which is involved in physiological role of exosomes is and  $\alpha$ -amino-3-  
6 hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors [50]. After releasing,  
7 exosomes can fuse with cell membrane of the recipient cell and release its constituents inside  
8 the cytoplasm of the cell, transfer lipid and protein content on the cell surface. Internalisation  
9 of exosomes can also take place by macropinocytosis or endocytosis process. The fusion of  
10 exosomes is regulated by receptor mediated mechanisms. These diverse mechanisms for  
11 transfer of constituents can initiate different signaling mechanisms in recipient cells [52].

12 Serotonin or 5-hydroxy tryptamine (5-HT) has also substantial role on the release of  
13 exosomes from the non-neuronal brain cells such as microglial cells. 5-HT is a major  
14 neurotransmitter which has important role in the pathophysiology of depression. According to  
15 monoamine theory level of 5-HT is decreased in depression and bipolar disorders [53].  
16 Serotonin can increase cytosolic levels of calcium ions, which leads to stimulation of release  
17 of exosomes from primary microglia [51]. Since 5-HT is involved in release of exosomes,  
18 their release is modified predominantly in case of depression. Both the factors discussed  
19 above i.e. cell to cell communication and neurotransmitter mediated release of exosomes are  
20 important aspects of psychopharmacology and involved in various CNS disorders including  
21 depression [54]. So exosomes can be considered to play a major role in etiopathogenesis of  
22 mental disorders given their prominence in the regulation of cell communication, and their  
23 regulation via neurotransmitters. In addition, signaling through one nerve cell to other nerve  
24 cell is important in synaptic plasticity [55].

### 25 **3. Role of Exosomes in Depression**

26 Depression is an important psychiatric disorder which is associated with disability and affects  
27 person daily life. Diagnostic and statistical manual of mental disorders, 4th edition (DSM-IV)  
28 has given 9 symptoms of depression out of which if 5 symptoms are present for more than  
29 two weeks then patient can be considered as depressed. However, these have certain  
30 limitations and more evaluation is required to confirm the depression cases. Multiple  
31 evidence gathered from previous research suggested that estimation of various biomarkers  
32 could be a useful approach for early detection of depression. Limited number of research

1 studies is conducted to evaluate the role of exosomes in mental disorders [56,57,58)  
2 Particular, Banigan et al. investigated exosomes from frozen postmortem prefrontal cortex  
3 to study alterations in miRNA in patients of psychoses and bipolar disorder. They found that  
4 miR-497 in psychoses patients and miR-29c in bipolar patients to be upregulated compared to  
5 control subjects [26]. This early piece work opens up interesting opportunities to study  
6 exosomes in mental disorders, demonstrating that miRNA cargo may be interesting to be  
7 evaluated in these phenotypes. The intercellular signalling of exosomes has important role in  
8 normal physiological and pathological conditions in the CNS. For example glial cell  
9 oligodendrocyte-derived exosomes are thought to be involved in neuroprotection against  
10 cellular stress. The reason of this protective effect of exosomes is due to involvement of  
11 proteins released from exosomes inside the recipient cell [56,59,60].

12 Recent data on signaling of exosomes in the CNS highlighted their role in transcriptional  
13 regulation, neurogenesis, plasticity, and neuroinflammation [60]. Exosomes delivered  
14 intravenously can be demonstrated to cross the BBB naturally. Exosomes from mesenchymal  
15 stem cells (MSC's) demonstrate anti-inflammatory and pro-growth effects in preclinical  
16 models and clinical cases reports and have been used intravenously and with intracerebral  
17 and intrathecal injection..

18 Among various biomarkers microRNAs (miRNAs) emerged as an important diagnostic  
19 marker that could be beneficial for patients to detect depression. These biomarkers have  
20 predominant role in pathophysiology of depression [61]. These molecules affect various  
21 molecular levels signaling pathways involved in progression of depression. miRNAs could be  
22 used as diagnostic and therapeutic biomarkers in MDD patients. Besides miRNAs, exosomes  
23 as nano- carriers could have been emerged as diagnostic biomarkers in various diseases such  
24 as MDD and other CNS disorders. Intravenous delivery of exosomes can able to cross the  
25 BBB [62]. Exosomes and MSC's demonstrate anti-inflammatory and pro-growth effects in  
26 preclinical models and clinical cases.

27 The exosomes released from mast cells have protective effect against increased oxidative  
28 stress. They have capacity to communicate a protective signal to other cells which are  
29 exposed to oxidative stress and this leads to reduction in cell death [63]. Exosomes that are  
30 produced by one cell under stress condition is able to induce capacity to tolerate increased  
31 oxidative stress in another cell that is known as recipient cell. This effect is linked with  
32 changed exosomal mRNA content that can be attenuated by reduced RNA activity through



1 exposure if UV light [64,65]. EVs and ROS are closely linked with oxidative stress. The pro-  
2 oxidant conditions are involved in release of EVs in fact, NADPH oxidase and nitric oxide  
3 synthase-2 (NOS-2) inhibitors inhibit the production of EVs in neutrophils [66,67].  
4 According to a study conducted by Wei ZX 2020 Exosomes obtained from patients with  
5 MDD caused depressive-like behaviors in mice with involvement of micro RNA regulated  
6 neurogenesis.

7 Intravenous injection of Exosomes derived from blood of MDD patients into normal mice  
8 exhibited the depressive phenotype behaviors as evaluated by the battery of behavioral tests  
9 such as forced swim test, tail suspension test, and novelty suppressed feeding tests, and these  
10 behaviours reversed by administration of exosomes derived from healthy subjects into  
11 chronic unpredictable mild stress (CUMS)-treated mice. [68]. In general, secretion of  
12 exosomes is increased in response to stress or pathological conditions [69].

13 .Various recent studies have observed changes in miRNAs in depressed patients, which target  
14 not only important pathways associated with memory function, synaptic plasticity but also  
15 formation of neurotrophic factors and modulation of immune cells[70,71]. Transfer of EVs  
16 or exosomes to nerve cells occurs through supporting cells of nervous system such as  
17 oligodendrocytes, microglia and astrocytes. Besides their potential as biomarkers, these  
18 exosomes have a predominant role in pathophysiology and dissemination of inflammatory  
19 pathways [72]. Reactive microglia were shown to release exosomes and microvesicles (MVs)  
20 carrying the pro-inflammatory cytokine interleukin-1 $\beta$  (IL-1 $\beta$ ), the IL-1 $\beta$ processing enzyme  
21 caspase-1, and the P2X7 receptor that may induce and propagate inflammatory reactions  
22 throughout the brain [73]. Additionally, monocytes that are activated by interferon alpha  
23 and/or lipopolysaccharides (LPS) release exosomes that carry altered miRNA profiles as  
24 shown in Fig. 1.

25 A study data published in the *Journal of Affective Disorders* indicates a link between higher  
26 levels of certain neuron-derived exosomes (NDE) and MDD [74]. In addition, a clinical study  
27 has been started to evaluate the safety and efficacy of exosomes against refractory depression  
28 [75]. Impaired adult hippocampal neurogenesis (AHN) is associated with depression and  
29 other neurodegenerative disorders like schizophrenia [76]. Protein analysis study reveals that  
30 exosomes has important role in modulation of adult neurogenesis [76]. In addition, injection  
31 of known pathogen containing exosomes into the dentate gyrus is sufficient to impair AHN in  
32 mice [77]. Cytokines and corticosteroids stimulate the release of glial cell astrocyte related

1 exosomes which contains several miRNAs that are critical for neurogenesis, stress response  
2 and survival of neurons [76]. So the exosomes may have dual role as it may be involved in  
3 maintenance and inhibition of neurogenesis in adult. Nerve cell to supporting glial cell  
4 signalling is one of the important mechanisms for exosomes [78]. Injection of exosomes in  
5 the tail vein of immune-challenged mice leads to increase in CNS expression of pro-  
6 inflammatory cytokine mRNA and associated miRNA in mice. Protein analysis of exosomes  
7 in the CNS reveals cargo involved in modulating adult neurogenesis [79].

#### 8 **4. Exosomes as Biomarker in CNS diseases and depression**

9 Exosomes have immense capability as diagnostic tool or biomarker for early diagnosis of  
10 CNS disorders such as Depression and Schizophrenia. Pathophysiological changes in  
11 contents of exosomes have been observed in CNS disorders. The exosomes can be isolated  
12 without much difficulty from biological fluids including urine, saliva and blood [80].  
13 Moreover exosomes contents are not easily degradable by biological fluids or enzyme in our  
14 body as they are protected inside the membrane [54]. In addition, *ex vivo*, these exosomes are  
15 stable for longer duration can be stored long time before analysis. It is possible to trace the  
16 surface marker indicating the origin for exosomes. The exosomes have great potential to  
17 cross BBB and this property makes them a good biomarker to identify a CNS disorder [81].  
18 Of particular interest is the ability to characterize exosomes based on their cell of origin,  
19 potentially providing an extra layer of insight into the disease of interest [82]. The  
20 significance of exosomes as diagnostic tool in disease like cancer is already established.  
21 However their role in CNS disorders like depression is yet to be established. Many of the  
22 circulating proteins and nucleic acids are diluted in the blood stream and the majority of them  
23 originate from other sources besides neurons due to the tight regulation of BBB in molecule  
24 transport.

25 Different types of exosomes (from multiple cells) can be segregated from biofluids using  
26 different segregation techniques like ultracentrifugation, immunomagnetic beads, and  
27 chromatography [83,84]. In addition, treatment with RNase, western blot analysis or mass  
28 spectrometry is also used for identification/segregation of exosomes contents. Various types  
29 of exosomes can be isolated from biofluids using multiple methods. Additionally, exosomes  
30 have phospholipid bilayer; therefore treatment with RNase prior to use will ensure that cargo  
31 used downstream was encapsulated within the vesicle [85]. Various types of exosomes may  
32 be identified using western blots or mass spectrometry of proteins which are involved in

1 biogenesis of ILVs, including tetraspanins and proteins involved in the ESCRT machinery  
2 needed for biogenesis [86]. It is essential to remember that these markers are not exclusively  
3 associated with exosomes and further characterizations of these markers are required..

4 Exosomes derived from developing and mature neurons of hippocampus, contain L1 cell  
5 adhesion molecule, and the GluR2/3 subunits of glutamate receptors, both of which are well  
6 established markers of nerve cells [87,88]. Protein markers, such as glial fibrillary acidic  
7 protein), glutamine aspartate transporter, and glutamine synthetase, can be used to enrich for  
8 astrocytic-derived exosomes. Additionally, myelin proteo-lipid protein and 2', 3'-cyclic  
9 nucleotide 3'-phosphodiesterase have been identified on oligodendrocytes derived from  
10 exosomes [89]. Neural-derived exosomes from plasma have also been used in a small study  
11 to evaluate protein biomarkers for patients with MDD [71]. The expression levels of  
12 exosomal miRNAs vary among different biological fluids in vivo and culture medium in  
13 vitro. So these changes in expression of exosomes in MDD are used to identify the early  
14 stage of disorder.

## 15 **5. Authors insight on the topic**

16 Utilization of EVs as drug carrier has been well explored in chronic disorders such as cancer  
17 and autoimmune disorders. In the future, drug loaded EVs could be explored to achieve  
18 maximum therapeutic benefit sparing normal healthy cells, with less off target mediated  
19 adverse effects of antidepressants with aim to target the selective affected region owing to  
20 MDD induced alteration in the CNS. Disorders like depression have lack of suitable  
21 diagnostic technique and treatment resistance is also more common. So, EVs maybe serve as  
22 as a potential therapeutic candidate as well as biomarker, which may be effective in the field  
23 of depression. For that, further studies have to be performed in order to understand the  
24 homing of EVs from different cell types in MDD. In MDD, it has been documented that in  
25 addition to abnormal neurotransmitter biogenesis and neurotransmitter shuttling, involvement  
26 of neuronal damage is highly prevalent condition during later stage of pathophysiological  
27 mechanism to disease progression. Neurogenesis capability of currently available  
28 antidepressants alone or in combination are being explored and evaluated in preclinical and  
29 clinical experiments. However, regenerative potential of these agents is not substantial to  
30 protect the vigorous constant neurodegenerative process during later stage of MDD and  
31 associated conditions. As it has been witnessed that EVs have regenerative ability and this  
32 exclusive property of EVs can be exploited to prevent the ongoing neurodegenerative process

1 of MDD. Cell therapy-based treatment especially induced pluripotent stem cells exhibit  
2 therapeutic activity in MDD and associated pathological conditions, however its usage for  
3 these conditions have been limited by its adverse effects such as graft rejection by recipient,  
4 immunogenicity reactions and low accessible and penetration to BBB. There are various  
5 approaches being explored to mitigate these adverse effects caused by cell therapy. In this  
6 context, there are advantages of EVs compared to cells such as reduced size, less  
7 immunogenicity, easier accessibility to BBB along with carrying some essential components  
8 of cell while mimicking benefits elicited by cell therapy of MDD. This lays foundation for  
9 cell-free therapy for MDD associated pathological condition. Despite the expected EV  
10 benefits for treating MDD, there are still unexplored uncertainties in terms of precise  
11 pharmacological mechanism of action, route of administration.

12 Selectivity and accumulation of EV to intended target site of action will be hampering the  
13 effort of EV as therapeutic potential in treating MDD. Though selective binding to its target  
14 can be achieved by producing EV which has selective binding surface expression molecules  
15 on its surface from genetically modified parental cells expressing the target selection marker.  
16 There are plenty of invitro cell-based assays need to be performed to achieve the tissue and  
17 target selective EV to maximize its therapeutic benefit sans adverse effect on healthy brain  
18 tissues. Another obstacle to developing EV for MDD would be owing to plethora of  
19 mechanism of action elicited by EV, narrow down a precise pharmacological molecular  
20 mechanism action can be difficult and challenging task. Nevertheless, the MDD disease  
21 pathway related molecular markers could be selected to elucidate the prime mode of  
22 mechanism action of EVs are warranted using various molecule biology tools (reporter,  
23 knock out or knock in based approaches. To address these issues, initiation of well-planned  
24 preclinical studies including high translational value based in vitro (Target binding,  
25 specificity, selectivity and internalization assays), pharmacokinetic assays (Tissue  
26 distribution studies with special emphasis on central nervous system using appropriate  
27 labelling agents) in vivo models of MDD, toxicology and safety pharmacology studies on  
28 vital systems should be warranted. In conclusion, owing to better advantage of EV compared  
29 to conventional treatment, future research EVs based therapy is expected to provide evidence  
30 on their potential as standalone therapy or in combination with standard antidepressants in the  
31 management of this devastating disease.

32

## 6. Conclusion

In the future, EVs may be useful as effective therapeutic carriers as well as biomarkers for the diagnosis of depression in early stages. However, further studies need to be done to prove the efficacy and pharmacokinetic profile of these exosomes. The exosomes have an advantage over conventional treatment to be able to cross the BBB. In depression modulation in levels of exosomes is taking place and this can be used as marker for detection of disorder in early stages. Chronic stress and inflammation also lead to modify the expression of exosomes. Exosomes are important in communication and signalling between two cells. These EVs also have role in synaptic plasticity and neurogenesis. However, some more detailed studies are required to validate the role of exosomes in MDD. In the future, exosomes may be used as effective treatment and biomarker for identification of depression and other CNS disorders.

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3 **Captions for Figures**

4 **Figure 1:** Microglia activation with release of extracellular vesicles (EVs).  
5 lipopolysaccharide (LPS), mitogen-activated protein kinases (MAPKs) superfamily, c-Jun N-  
6 terminal kinase (JNK 1/2) and p38 proteins, nuclear factor- $\kappa$ B (NF- $\kappa$ B) interleukin (IL)-1 $\beta$ ,  
7 IL-6 and tumour necrosis factor (TNF)- $\alpha$ .

8 **Captions for Graphical Abstract**

9 Biosynthesis and release of extracellular vesicles:

10