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# A systematic review of secretome-based therapies for Alzheimer's disease: Bridging the preclinical and clinical gap

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**ABSTRACT**

Alzheimer's disease (AD) is a progressive neurodegenerative disorder with a rapidly increasing prevalence. Current therapeutic options primarily manage symptoms, not modifying the disease. Secretome-based therapies have emerged as a promising avenue for AD treatment in targeting multiple pathways and promoting neuroprotection and regeneration. This systematic review evaluated the preclinical and clinical evidence for secretome-based therapies in AD. A systematic search was conducted across Scopus, PubMed, ScienceDirect, and Cochrane Library. The Systematic Review Protocol for Animal Intervention Studies risk of bias (RoB) tool was used for preclinical studies and Cochrane RoB 2.0 for clinical studies. We performed a qualitative analysis of the study results. Included 21 in vivo studies and 2 clinical trials revealed promising outcomes of treatments involving secretomes, exosomes, and extracellular vesicles from different cell sources. The therapies could reduce amyloid plaque load, reactive gliosis, and enhance neuronal density. These findings suggested the treatments reveal mechanisms of action in neuroprotection, neuroregeneration, and inflammation modulation, which are critical in AD pathology. Ongoing trials also supported the safety and efficacy of the treatment strategies. However, translational medical study faces several challenges regarding large-scale production, optimization of protocols, and understanding biomarkers. The heterogeneity in secretome-based therapy administration has complicated the comparison of study outcomes and the translation of preclinical findings into clinical settings. A deeper understanding of the secretome's mechanisms of action, optimal dosing, and delivery methods are needed to maximize therapeutic outcomes. Despite secretome-based therapies holding significant promise for AD treatment, addressing the identified gaps and limitations is crucial for advancing these therapies from preclinical research to clinical practice.

## INTRODUCTION

Alzheimer's disease (AD) is a neurological condition that causes cognitive deficits to progress to the point where a person is unable to perform daily activities. It is the most common form of dementia, accounting for 60%–70% of all dementias [1]. The prevalence of AD is increasing rapidly and is projected to reach 16 million individuals by the year 2050 [2]. Dementia is expected to affect up to 24 million people worldwide, and its

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incidence is expected to rise every 20 years until at least 2040 [3]. The prevalence of AD increases exponentially with age, particularly after 65 years [3]. The burden of AD is significant, with a prediction for worsening trends in the United States and other countries [4]. Of the about 55 million people worldwide with dementia, 60%–70% are estimated to have AD [1]. The prevalence of dementia in adults 60 years of age and older was estimated to be 3.9% worldwide, with regional prevalences of 1.6% in Africa, 4.0% in China and the Western Pacific, 4.6% in Europe, and 8.0% in North America [1,5]. According to the Alzheimer's Association, about 6.5 million adults 65 years of age and older have AD In the United States. Of these, almost 70% are 75 years and older [5].

Early diagnosis of AD is crucial for several reasons, including providing timely support and care, reducing the financial burden on healthcare systems, and possibly decreasing the progression of the disease [6]. The diagnosis rate for AD remains low, and there is an urgent need to improve diagnosis rates so that those at greatest risk can be identified [6]. The urgency of AD is also underscored by the growing body of research on risk factors and the need for effective prevention strategies [7,8]. Besides, there are currently only two fully approved non-modifying-disease therapies for AD, including N-methyl d-aspartate receptor antagonists and acetylcholinesterase inhibitors [9]. The FDA granted partial approval for Aducanumab and Lecanemab, two monoclonal antibodies that target amyloid [10–12]. However, these regiments have been questioned for their efficacy because of the significant risks of amyloid-related imaging abnormalities, such as hemorrhage or edema [13,14]. Furthermore, this class of drugs has no direct curative effect in AD [15].

Alzheimer's is caused by neuronal death, which covers a large area of the central nervous system and is stimulated by the plaques formed by the deposition of amyloid-β (Aβ) peptides [16]. Insoluble Aβ fibrils are produced as a result of modified cleavage of the amyloid precursor protein (APP) by β- and γ-secretases, which then Insoluble Aβ fibrils oligomerize and interfere with synaptic signaling, contributing to neurodegeneration [17,18]. Additionally, the deposition of Aβ in the brain and the presence of NFTs lead to the gradual loss of synapses and impair mitochondrial function, cognition, and intracellular neurofibrillary tangles memory [17,19]. Other factors such as insulin resistance, oxidative stress, impaired energy metabolism, and the pathophysiology of AD are also linked to the activation of the inflammasome complex [17]. AD pathogenesis is characterized by a complex system of molecular and cellular mechanisms and involves multiple interconnected pathways, making it a challenging area for research and the development of effective treatments [20].

Additionally, it has been discovered that extracellular-vesicles (EVs) in the secretome contribute to the pathophysiology of AD, with EVs inducing pro-inflammatory effects in mixed cortical cultures [21]. The chemical composition of mesenchymal stem cell (MSC)-derived secretome, stem cell-derived exosomes, and EVs includes a variety of bioactive molecules that contribute to their therapeutic potency against AD. The MSC-derived secretome comprises soluble factors such as cytokines, chemokines, growth factors (e.g., VEGF, NGF, and BDNF), and extracellular matrix proteins, which collectively promote neuroprotection, neuroregeneration, and immunomodulation [22,23]. Stem cell-derived exosomes, a subtype of EVs, are enriched with proteins (e.g., tetraspanins and heat shock proteins), lipids (e.g., sphingomyelin and cholesterol), and nucleic acids (e.g., miRNA and mRNA) that facilitate intercellular communication and modulate inflammatory responses, oxidative stress, and Aβ aggregation [24–27]. These exosomes can cross the blood-brain barrier (BBB), delivering their cargo directly to neural cells, thereby reducing neuroinflammation, enhancing neurogenesis, and improving cognitive functions in AD models. Overall, the combined action of these bioactive components in MSC-derived secretomes and exosomes makes them potent candidates for AD therapy by targeting multiple pathological mechanisms simultaneously [28,29]. Previous studies of AD animal models have shown that the secretome derived from MSCs could reduce the amount of amyloid plaque and reactive gliosis, as well as increase hippocampal and cortical neuronal density, indicating potential positive effects on AD pathology [30,31].

Preclinical studies and clinical trials have highlighted the safety, disease-specific therapeutic potential, and neuroprotective effects of MSC secretome for AD treatment [22,32]. However, there are challenges and limitations, including the need to understand the impact of bioengineering advances, the development of large-scale good manufacturing protocol (GMP) secretome-based products, and the optimization of secretome-based therapy for clinical use [33]. Despite these challenges, secretome-based therapy shows promise as a potential treatment for AD, as evidenced by preclinical studies and ongoing clinical trials. Thus, this study aimed to determine information gaps comprehensively by evaluating the preclinical and clinical data for secretome-based therapy, including exosome and microvesicles in AD.

## METHODS

### Study design

This systematic review was carried out based on the Systematic Review Protocol for Animal Intervention Studies (SYRCLE). The systematic review protocol has been registered in PROSPERO (ID: CRD42024498742). We carried out a thorough search of academic databases, including Scopus, PubMed, ScienceDirect, and the Cochrane Library. Keywords generated from free texts and medical subject headings were combined in the search strategy (Table 1). We also searched by previous references of related review articles.

### Eligibility criteria

The inclusion criteria of this study were *in vivo* and clinical studies focused on stem cell-based therapy through the secretome, exosomes, and microvesicles. We also restricted the article language to English. Incompatible results were excluded and we also did not include review, case, or editorial studies.

### Study selection

The results of the search were exported to rayyan.ai. After removing duplicate studies, the articles were examined

**Table 1.** The search strategy for eligible articles.

| Database | Search strategy |
|---|---|
| Scopus | (“Alzheimer's Disease” OR “Alzheimer Dementia” OR “Senile Dementia” OR “Alzheimer Sclerosis” OR “Alzheimer Syndrome”) AND (Secretome OR Exosomes OR Microvesicles) AND (Neuroprotection OR “Neural Protection” OR “Neuronal Protection” OR Neuroregeneration OR “Neural Regeneration” OR “Neuronal Regeneration” OR Therapeutic OR Therapy OR Treatment) |
| | Filters (Limit-to): Document type “*Articles”;* Language *"English"* |
| PubMed and cochrane library | (“Alzheimer's Disease” OR “Alzheimer Dementia” OR “Senile Dementia” OR “Alzheimer Sclerosis” OR “Alzheimer Syndrome”) AND (Secretome OR Exosomes OR Microvesicles) AND (Neuroprotection OR “Neural Protection” OR “Neuronal Protection” OR Neuroregeneration OR “Neural Regeneration” OR “Neuronal Regeneration” OR Therapeutic OR Therapy OR Treatment) |
| Sciencedirect | ("Alzheimer's Disease" OR "Alzheimer Dementia") AND (Secretome OR Exosomes OR Microvesicles) AND (Therapeutic OR Therapy OR Treatment) |
| | Filters (Limit-to): Article type “*Research article”* |

by the titles and abstracts. Full-text of records were retrieved and screened based on eligibility criteria. The articles were independently reviewed by two reviewers and a third reviewer was used in any disagreements.

### Quality assessment

The SYRCLE risk of bias (RoB) tool was used to measure the quality of the pre-clinical studies and Cochrane RoB 2.0 was used for clinical studies. Critical judgment was conducted by two reviewers and a third party was included if there were any disagreements. Traffic-light plot graphs were used to display the results of the RoB assessment, demonstrating whether risks were low, high, or unclear.

### Data extraction and analysis

Data extraction was independently performed by three reviewers using predefined sheets that included the following information: general information regarding the authors, study design, subject’s characteristic data, and outcomes related to the efficacy and safety of secretome-based therapy in AD. We included outcome measures of immunological assays (Immunocytochemical analysis, Multielectrode array recording, Real-time polymerase chain reaction, Western blot, Immunohistochemistry, Immunofluorescence analysis, and so on), electron microscopy analysis, and behavior analysis. The data were analyzed qualitatively.

## RESULTS

### Study selection

A systematic search was conducted from 4 databases resulting 660 records. After the removal of duplicated articles, we screened 465 articles for eligible title and abstract, and 421 articles were excluded. From 44 articles, a total of 36 full-text retrieved articles were assessed for eligibility criteria. Finally, we included 21 *in vivo* studies, with details of 4 secretome studies, 13 study of exosomes studies, and 4 micro-vesicles studies (Fig. 1). In addition, we included 2 clinical studies, which mentioned from the eligible articles.

### RoB assessment

The quality of the included studies was evaluated using the RoB methodology for animal research created by SYRCLE

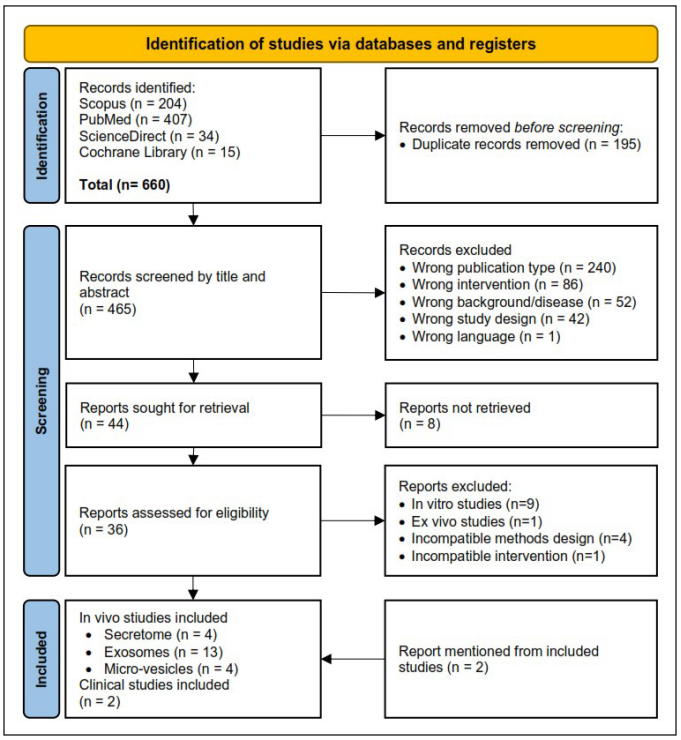


**Figure 1.** PRISMA flowchart diagram.

[34]. Ten criteria were evaluated to determine whether the RoB was low, high (if information was lacking), or unclear (if not enough information was available). A set of ten criteria were assessed to ascertain if the RoB was low (if details met the criteria), high (if information was lacking), or unclear (if not enough information was available). Based on the criteria assessment, there were a significant risk for randomization of sequence generation, housing, and outcome assessment with studies percentages of 71%, 76%, and 52%, respectively. Furthermore, the blinding of housing and outcome were lack of reporting, with percentages of 90%–71%, respectively. However, other criteria were at low RoB (Fig. 2).For clinical studies, Cochrane RoB 2.0 was used by measuring five domains and performed as per the prescribed algorithm [35]. We found that both studies have a high risk for the

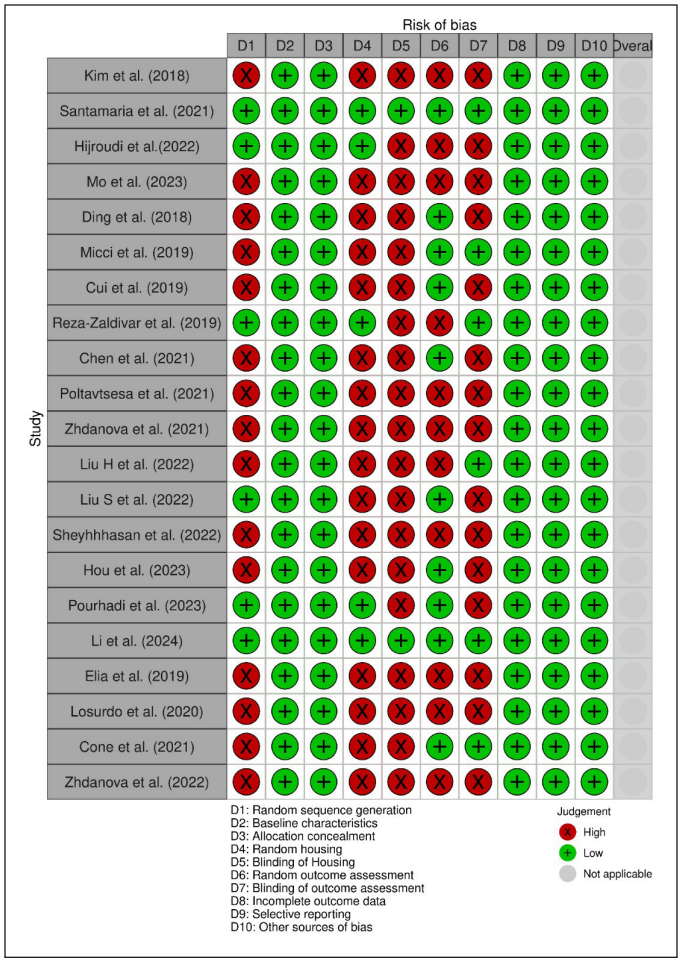


**Figure 2.** Result of SYRCLE RoB assessment.

randomization process as they were open-labeled studies. RoB for the other four domains was low (Fig. 3).

### Characteristics of animal studies

This systematic review included studies varying from 2018 to 2024. Study characteristics of *in vivo* and clinical studies were described in Table S1. Based on the results, different animal models were used in eligible studies, which were APP/PS1mice ($n$ = 5), 5XFAD mice ($n$ = 4), NMRI mice ($n$ = 3), C57BL/6 mice with induction ($n$ = 3), Wistar rats ($n$ = 2), BALB/c mice ($n$ = 1), triple-transgenic AD mice ($n$ = 1), APP/PS1/SIRT1 CKO mice ($n$ = 1), Nestin-δ-HSV-TK mice ($n$ = 1), and J20 mouse model of AD ($n$ = 1). The ages of animal models were ranged from 4 weeks to 22 months old. Male animal model was more often to be used ($n$ = 12) than female ($n$ = 2) or both sexes ($n$ = 2). Induction was performed in non-genetical engineered animal using streptozotocin ($n$ = 3) and Aβ aggregates or Aβ1–42 ($n$ = 2).

### Characteristics of clinical trials

From 21 included articles, two of which referred for further clinical trials as shown in Table 2. Both studies were designed as open-label, phase I/IIa clinical trial. Each study required AD patients, which were 9 patients, with age above 50 years old. A study by Kim *et al.* [36] performed intervention for three patients by low dose ($1.0 \times 10^7$ cells/2 ml), and six patients by high dose ($3.0 \times 10^7$ cells/2 ml) of human umbilical cord blood (hUCB)-derived MSCs. For all patients, three consecutive MSC injections were given at 4-week intervals. After the first hUCB-MSC injection, these patients were monitored for up to 12 weeks. In the extended observation phase, they were monitored for an additional 36 months.Click or tap here to enter text. However, a study by Xie *et al.* [37] used nasal spray devices to deliver allogenic human adipose-derived MSCs exosomes (ahaMSCs-Exos) ($2 \times 10^8$, $4 \times 10^8$, and $8 \times 10^8$ particles) dissolved in saline (1 ml) twice a week for a total of 24 sessions [37]. Furthermore, the 3+3 design was applied in the three groups, which were split into low, medium, and high interventional dose groups.Click or tap here to enter text.

### Data analysis of *in vivo* study results

Table S2 provides results summary from *in vivo* studies related to secretome-based therapies for AD. We include information on various parameters and outcomes assessed in these studies, such as cognitive performance, spatial memory, therapeutic efficacy, and brain region-specific effects. The results present data from a total of 21 *in vivo* studies, including 4 secretome studies, 13 exosomes studies, and 4 micro-vesicles studies. The studies varied in terms of the type of secretome-based therapy used, the administration route, and the specific outcomes measured. Some of the key findings reported in the table include improved spatial memory, enhanced cognitive performance, regulation of neuronal and astrocytic activity, and prevention of spatial memory deterioration.The study of secretome-based therapy explored the therapeutic potential of various types of secretomes derived from MSCs, neural stem cells (NSCs), and induced pluripotent stem cells in animal models of AD. These studies demonstrated promising results in mitigating AD symptoms and pathology through different mechanisms. For instance, Kim *et al.* [38] reported that hUCB-MSCs could rescue synaptic density loss induced by Aβ42 peptide *in vivo*, highlighting the protective effect of hUCB-MSCs against synaptic dysfunction mediated by thrombospondin-1 (TSP-1). Click or tap here to enter text. Santamaria *et al.* [39] observed memory recovery and a reduction in amyloid plaques in APP/PS1 mice following a single intravenous injection of MSC-derived conditioned serum (MSC-CS), suggesting that MSC-CS mimics the neuroreparative effects of MSCs through paracrine action.Click or tap here to enter text. Hijroudi *et al.* [40] found that NSCs conditioned medium (NSCs-CM) improved memory retention and reduced Aβ plaque formation in AD mice, indicating that NSCs-CM supports neuronal survival and function.Click or tap here to enter text. Finally, Mo *et al.* [41] showed that intranasal delivery of iPSC-derived central nervous system cells secretome (CNSC-SE) improved deficits in cognitive function and spatial memory in 5xFAD mice, with CNSC-SE promoting cortical neuron differentiation and reducing amyloidosis. These studies collectively underscore the potential of secretome-based therapies in addressing various aspects of AD pathology, including synaptic dysfunction, amyloidosis, neuroinflammation, and cognitive decline, through multiple mechanisms of action.

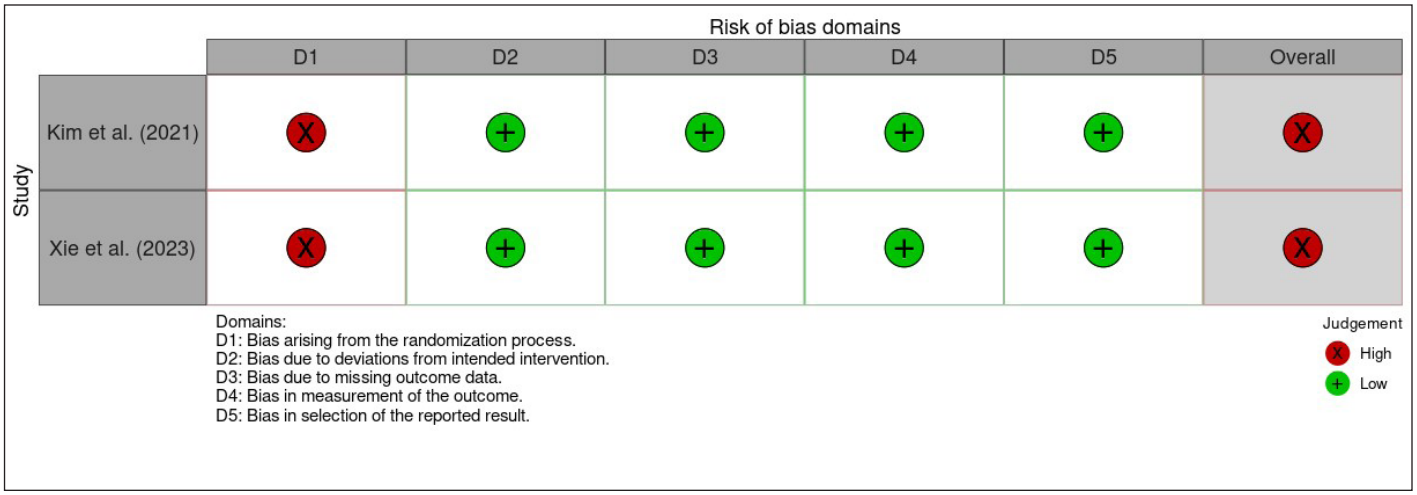


**Figure 3.** Result of cochrane RoB 2.0. assessment.

**Table 2.** Characteristics of clinical trials.

| Author (Year) | Study type/ design | ID | Participant & sample size | Age | Intervention |
|---|---|---|---|---|---|
| Kim *et al.* [36] | Open-labelled Phase-I clinical trial | NCT03172117 | AD dementia patients ($n$ = 9) | 50–85 years | Intracerebroventricular injection of hUCB-derived MSCs ($1.0 \times 10^7$ and $3.0 \times 10^7$ cells/2 ml) |
| Xie *et al.* [37] | Open-labelled Phase-I/IIa clinical trial | NCT04388982 | Mild or moderate AD ($n$ = 9 for safety analysis and $n$ = 8 for efficacy analysis) | ≥50 years | intranasal administration of ahaMSCs- Exos ($2\times10^8$, $4\times10^8$, $8 \times 10^8$ particles) |

In the study that described exosomes-based therapy, there were various tests and assays used to evaluate the efficacy of the exosome treatments, such as the Morris Water Maze test for learning and memory, immunostaining for cellular and molecular analysis, and Western Blot and ELISA for protein quantification. Most of the exosomes-studies highlighted decreased Aβ plaque deposition, enhancements of cognitive function, and changes in neuronal and synaptic markers that indicates neuroregeneration or synaptic plasticity [24,42–53]. In terms of action mechanism, these studies showed insights into how these exosome-based therapies might be working at a molecular level. Exosomes have been shown to modulate microglial activation states, reduce inflammation, and decrease levels of Aβ, which are implicated in AD pathology. Exosomes-based therapies also promoted neurogenesis, enhanced mitochondrial biogenesis, and activate signaling pathways like SIRT1-PGC1α, which are beneficial for neuronal health and function. Thus, exosome-based therapies have potential therapeutic actions in AD models, with effects on learning and memory, synaptic plasticity, and neuroinflammation.However, the study of EVs-based therapy derived from MSCs also demonstrated to be therapeutic agents for AD. The studies detailed in the table explore the potential benefits of administering MSC-derived EVs to animal models of AD. For instance, Elia *et al.* [16] found that administering bone marrow-derived MSC-EVs (BM-MSC-EVs) to APP/PS1 mice reduced AD pathology, including Aβ plaque area and dystrophic neurites. The proposed mechanism is that these EVs carry neprilysin, an enzyme that degrades Aβ, and inherit anti-inflammatory and neurotrophic properties from their parental BM-MSCs.Click or tap here to enter text. Similarly, Losurdo *et al.* [54] observed a decrease in microglia activation and an increase in the hippocampal dendritic spine density and other brain regions after treating mice with MSC-derived EVs, suggesting immunomodulatory and neuroprotective effects.Click or tap here to enter text. Cone *et al.* [55] reported improved cognitive performance and reduced Aβ plaque load in EV-treated mice, with the EVs exhibiting immunoprotective and immunomodulatory abilities and the capacity to cross the BBB.Click or tap here to enter text. Finally, Zhdanova *et al.* [56] demonstrated that intranasally administered vesicles could reach the hippocampus and neocortex, acting as nanocontainers for targeted delivery of compounds to brain regions affected by neurodegeneration. Click or tap here to enter text. These findings collectively suggested that MSC-derived EVs could be a promising Alzheimer's therapeutic approach, offering benefits such as Aβ plaque reduction, cognitive performance improvement, and neuroprotection. These studies also highlight the potential mechanisms by which these effects are achieved, including direct Aβ degradation, immunomodulation, and the facilitation of cell-to-cell communication across the BBB.

Overall, the included studies demonstrated that treatments involving secretomes, exosomes, and EVs from different cell sources can significantly improve cognitive functions, reduce amyloid plaque deposition, and modulate neuroinflammation in AD animal models. The mechanisms of action involved modulation of signaling pathways related to neuroprotection, neurogenesis, synaptic plasticity, and reduction of Aβ levels. Intranasal and intravenous administrations were also noted for their effectiveness while transporting these therapeutic agents to the brain, demonstrating the potential of secretomes-based therapies as promising strategies for AD managements.

### Safety and efficacy of MSCs for AD based on clinical studies

Table 3 showed a detailed comparison of the safety and efficacy of MSCs in treating AD, based on two distinct studies. The study by Kim *et al.* [36] reported several adverse events including fever in 9 participants, headache in 7, nausea in 5, and vomiting in 4, all of which subsided within 36 hours. Furthermore, two participants experienced three severe adverse events that were thought to be related to the experimental drug. Nevertheless, no dose-limiting toxicities were seen. Interestingly, five individuals finished a 36-month extended observation trial without experiencing any more severe side effects, indicating a long-term safety profile. However, the efficacy data for this study was not reported (N/R) [36]. Besides, the study by Xie *et al.* [37] in 2023 demonstrated a more promising outlook in terms of both safety and efficacy. No adverse events were reported, indicating the safety and tolerability of the treatment. The AD Assessment Scale-Cognitive section (ADAS-cog) scores in the medium-dose group showed improvements in cognitive function, with a 2.33-point reduction from the baseline, showing a decrease in cognitive deterioration. This suggested that the treatment was effective. Furthermore, there was a 2.38-point rise in the baseline Montreal Cognitive Assessment (MoCA-B) basic version scores, indicating improved cognitive function. The continuous improvement in ADAS-cog scores by 3.98 points until week 36 further supported the sustained cognitive benefits of the treatment. Despite the fact that the three dose groups did not significantly differ in terms of changed amyloid or tau deposition, the medium-dose arm exhibited less neurodegeneration, indicating potential neuroprotective effects [37].

## DISCUSSION

Mesenchymal stromal cell-derived secretomes, which includes the use of exosomes and EVs, represents a novel and promising approach in the treatment of AD, an progressive neurodegenerative condition marked by memory loss and cognitive impairment [57]. We offer a comprehensive evaluation of the possible benefits of secretome-based therapies for AD. We highlight that secretome-based therapies, which include a complex mixture of proteins, nucleic acids, and lipids secreted by cells, have shown promise in targeting multiple disease pathways, promoting neuroprotection, and regeneration based on animal studies. Furthermore, a recent clinical trial suggested a reduction of cognitive decline and sustained cognitive benefits.Click or tap here to enter text.Secretome-based therapies, particularly those derived from MSCs, exert their effects through paracrine mechanisms that can modulate the microenvironment of the CNS. These therapies have been shown to promote neurogenesis, reduce oxidative stress, alleviate cognitive impairment, and increase the number of

**Table 3.** Safety and efficacy of MSC for AD.

| Reference | Safety | Efficacy |
|---|---|---|
| Kim *et al.* [36] | • Adverse events : fever ($n$ = 9), headache ($n$ = 7), nausea ($n$ = 5), and vomiting ($n$ = 4) (subsided within 36 hours).<br>• Three serious adverse events in two participants (considered to be related to the investigational product, but no dose-limiting toxicities).<br>• Five participants completed a 36-month extended observation study without further serious adverse events. | N/R |
| Xie *et al.* [37] | The trial reported no adverse events, indicating that the treatment was safe and well-tolerated. | Cognitive Function Improvement<br>• A decrease in ADAS-cog scores by 2.33 (1.19) compared with the baseline, suggesting reduced cognitive decline.<br>• An increase in the basic version of MoCA-B scores by 2.38 (0.58) compared with the baseline.<br>• Continuous improvement in ADAS-cog scores by 3.98 points until week 36, further supporting the sustained cognitive benefits of the treatment.<br>• Neuroprotection Indicators<br>• Although there were no significant differences in altered amyloid or tau deposition among the three dosage arms, the medium-dose arm exhibited less shrinkage in hippocampal volume, hinting at a degree of neuroprotection |

neuroblasts in the hippocampus region, which are crucial for memory and learning [22]. The MSC-derived secretome contains a variety of bioactive molecules (nerve growth factor and brain-derived neurotrophic factor), which are vital for neuronal survival and function [22,58,59]. However, stem cell-derived exosomes have also been found to reduce the load of Aβ plaque formation, inhibit neuronal death, and promote neurogenesis, thereby potentially ameliorating the cognitive deficits associated with AD [45,60]. Additionally, they might change the pro-inflammatory to anti-inflammatory phenotypes of microglia, which contributed reduce neuroinflammation as a key component of AD pathology [60]. Additionally, extracellular vesicles, a key component of the secretome, can mediate the propagation of tau aggregation and decrease Aβ plaques, addressing two major pathological hallmarks of AD [58,61]. Overall, included preclinical studies of this systematic review showed improvements in cognitive functions, reduced amyloid plaque deposition, and modulated neuroinflammation. Intranasal and intravenous administrations have been effective in delivering these therapeutic agents to the brain.Clinical trials have shown the safety and long-term safety profile of MSC secretome-based therapies, with some adverse events subsiding within 36 hours in intracerebroventricular injection [36,37]. But, clinical trial by Xie *et al.* [37] revealed no signficant changes in the accumulation of tau or amyloid among different dosage arms, although the medium-dose arm showed less hippocampal volume shrinkage, hinting at neuroprotection.Click or tap here to enter text. These two clinical trials were utilized MSCs derived therapy through intranasal and intracerebroventricular administrations. The results demonstrated that intranasal administration may provide lower adverse effects of the therapy. Compared to other developing therapies like gene therapy and small molecule drugs, secretome-based therapies can address multiple AD pathology aspects simultaneously. Due to their nanoscale size, these therapies are considered to have a higher safety profile, potentially offering a cell-free therapy option that could circumvent the risks related to the direct transplantation of cells, such as immune rejection and tumor formation [62]. In this systematic review, several challenges were addressed to bridge the gap between animal studies and clinical trials effectively. Differences in disease pathology between animal models and humans, as well as the need for well-designed clinical trials to assess therapeutic outcomes accurately, are critical challenges that need to be resolved [63]. A deeper understanding of how MSCs and their secretome exert their effects is crucial for optimizing therapeutic strategies and identifying biomarkers for treatment efficacy. Producing MSC-derived secretome, exosomes, and EVs in quantities sufficient for clinical trials while ensuring batch-to-batch consistency is challenging. Standardization of production methods, isolation, characterization, dosage, and route of administration is crucial for translating preclinical success into clinical settings [62]. The use of stem cells and their derivatives also faces regulatory and ethical scrutiny, varying significantly across countries. Ensuring compliance with regulatory requirements is essential for advancing these therapies from the laboratory to the clinic [64]. However, these challenges require extensive clinical validation to establish safety, efficacy, and practicality as an AD treatment option.This systematic review also acknowledged several limitations, including the high RoB in randomization process and outcome assessment in animal studies, as well as the randomization process in clinical studies due to their open-label design. We also highlighted the paucity of existing clinical evidence for secretome-based therapies in AD and the challenges in translating preclinical findings into clinical applications, such as the need for bioengineering advances and the development of large-scale GMP products. It may be difficult to synthesize data and reach strong conclusions regarding the safety and efficacy of secretome-based therapeutics due to the heterogeneity in study designs and results among the included studies. Further research and development are needed to address current challenges and advance these therapies towards clinical application.

## CONCLUSION

Secretome-based therapies represent a promising frontier in the treatment of AD, which also involving exosomes and extracellular vesicles, in reducing amyloid plaque load, reactive gliosis, and enhancing neuronal density. These outcomes suggest mechanisms of action in neuroprotection, neuroregeneration, and inflammation modulation, which are critical in AD pathology. This therapeutical approach faced several challenges in translating preclinical findings into clinical settings, including the need for large-scale production, optimization of protocols, understanding biomarkers, and addressing the heterogeneity in administration methods. Despite these challenges, we highlighted that secretome-based therapies hold significant promise for AD treatment, emphasizing the need for further research and development to address the identified gaps and limitations.

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## AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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## CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

## ETHICAL APPROVALS

Not applicable.

## DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

## PUBLISHER'S NOTE

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## USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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## SUPPLEMENTARY MATERIAL

The supplementary material can be accessed at the journal's website: [https://japsonline.com/admin/php/uploadss/4396_pdf.pdf].