Ind. J. Pharm. Edu. Res., 2025; 59(2):767-776.
https://www.ijper.org

Original Article

Comparative Efficacy of Cytokines and Drugs in Promoting the Expansion of Bone Marrow-Derived Mesenchymal Stem Cells: A Systematic Review and Meta-Analysis

Jinhui Liu[1], Sheng Liu[2], Chunming Li[3,*]

[1]Department of Oral Restoration, Dalian Stomatological Hospital, Dalian, CHINA.
[2]Department of Oral and Maxillofacial Surgery, Dalian Stomatological Hospital, Dalian, CHINA.
[3]Department of Oral and Maxillofacial Surgery, Second Affiliated Hospital of Harbin Medical University, Harbin, CHINA.

ABSTRACT

Aim/Background: Bone Marrow (BM) Mesenchymal Stem Cells (MSCs) possess significant clinical potential, yet their limited expansion ability in the laboratory restricts their broad application. While cytokines and pharmaceuticals are commonly used strategies to promote BM-MSC expansion, a systematic comparison of their effects has not been conducted. This study aims to evaluate and compare the effects of cytokines and pharmaceutical agents on the proliferation and osteogenic differentiation of BM-MSCs through a comprehensive review and meta-analysis. **Materials and Methods:** A comprehensive search was performed across PubMed and Web of Science to identify 15 quantitative studies comparing the efficacy of cytokines or pharmaceutical agents against control groups in enhancing BM-MSC proliferation or osteogenic differentiation. A meta-analysis was conducted to assess the pooled effect sizes of cytokines and pharmaceuticals. **Results:** Meta-analysis revealed a significant enhancement in BM-MSC proliferation with both cytokines (SMD=1.42) and drugs (SMD=1.28), as well as in osteogenic differentiation (SMD=1.65 and 1.51). Subgroup analysis identified specific cytokines, such as LIGHT, IL-17A, IL-6, TGF-β1, BMP-2/7, and drugs including melatonin, HDAC inhibitors, AMPK activators, and artemisinin, as having significant promoting effects on BM-MSC expansion. **Conclusion:** This research validates the efficacy of both cytokines and pharmaceutical agents in enhancing BM-MSC expansion, despite limitations such as heterogeneity in study design and reporting quality. The findings provide valuable insights for fundamental and translational BM-MSC research. Future studies could explore the combined use of these two methods to develop safer and more effective strategies for increasing BM-MSC numbers.

Keywords: Bone Marrow-Derived Mesenchymal Stem Cells, Cytokines, Drugs, Proliferation, Osteogenic Differentiation, Systematic Review, Meta-Analysis.

Correspondence:
Dr. Chunming Li, MD
Department of Oral and Maxillofacial Surgery, Second Affiliated Hospital of Harbin Medical University, 246 Xuefu Road, Nangang, Harbin, CHINA.
Email: 13314519977@163.com

Received: 12-06-2024;
Revised: 03-10-2024;
Accepted: 23-01-2025.

INTRODUCTION

Bone Marrow (BM) derived Mesenchymal Stem Cells (BM-MSCs) have gained significant popularity because of their unique properties including self-renewal, differentiation into multiple cell types and Immune Modulating Properties (Figure 1).[1] These intrinsic properties position BM-MSCs as potential frontrunners for a wide array of medical uses, including tissue regeneration, cellular treatments, and interventions for immune and inflammatory conditions.[2] However, the constrained yield of BM-MSCs attainable from bone marrow aspirates represents a significant hurdle to their clinical application.[3] To surmount this challenge, extensive investigations have been undertaken to enhance the *ex vivo* expansion of BM-MSCs while preserving their stemness and functional properties.[4]

Among the various strategies employed to enhance the expansion of BM-MSCs, the utilization of cytokines and small molecule drugs has shown promising results.[5,6] Bone Marrow derived Mesenchymal Stem Cells (BM-MSCs) receiving various cytokines such as LIGHT, IL-17A, IL-6, TGF-β1 and BMP2/7 through enhanced multiple signaling pathways show promotion growth and specialization.[7-10] Similarly, small molecule drugs, including melatonin, Histone Deacetylase (HDAC) inhibitors, AMP-activated Protein Kinase (AMPK) activators, and artemisinin, have demonstrated the ability to enhance BM-MSC expansion and modulate their differentiation potential.[11-16]

Although there is an increasing amount of evidence backing the use of cytokines and drugs to enhance BM-MSC proliferation,[17-22]

DOI: 10.5530/ijper.20251689

a thorough analysis comparing their effectiveness and the mechanisms involved is still lacking. Due to the absence in prior studies, we attempt to fill this void by synthesizing the available data about the effect of cytokines and medications on the proliferation and differentiation of Bone Marrow derived Mesenchymal Stem Cells (BM-MSC). This review seeks to guide future studies and enhance the development of efficient protocols for expanding BM-MSCs by providing a thorough overview of the current research landscape and highlighting the most promising approaches, thereby accelerating their clinical use in regenerative medicine.

Methods Characteristics of BM-MSCs

Literature search strategy

Search database and time range

The literature search for this systematic review was conducted in two widely used biomedical databases, specifically PubMed and Web of Science. PubMed is the service provided by the National Center for Biotechnology Information (NCBI) which manages a vast amount of life science literature as well as biomedicine. Conversely, Web of Science, a robust academic repository furnished by Clarivate Analytics, integrates top-tier literature spanning diverse disciplines. The selection of these databases for the literature exploration guarantees thorough inclusion of pivotal articles pertinent to the research subject matter.

The literature search encompassed the inception of each database through May 2024 to encompass all pertinent published studies. In PubMed, the search period spanned from 1966 to May 2024, while in Web of Science, it extended from 1900 to May 2024. The broad time frame was chosen to ensure a thorough compilation of historical and contemporary research findings, providing a solid literature foundation for the systematic review.

To ensure currency and relevance, multiple searches and screenings were performed in the PubMed database on May 26, 27, and 28, 2024. This effort yielded 40 articles related to cytokines and 43 articles related to drugs. Furthermore, a search was executed in the Web of Science database on May 30, 2024, revealing a total of 1,389 pertinent articles. These iterative search and screening procedures ensure the integration of the most recent and germane literature into the systematic review.

Search formula construction

The development of the search methodology was grounded in the research inquiry and the PICOS (Participants, Interventions, Comparators, Outcomes, and Study types) structure. The primary search keywords included "mesenchymal stem cells from bone marrow," "cell signaling proteins," "pharmaceuticals," "cell growth," and "cell specialization." These keywords were integrated with Boolean logic (AND, OR) and tailored for each specific database.

For PubMed, the search strategy was as follows: ("cytokin"[All Fields] OR "cytokine s"[All Fields] OR "cytokines"[MeSH Terms] OR "cytokines"[All Fields] OR "cytokine"[All Fields] OR "cytokinic"[All Fields] OR "cytokins"[All Fields] OR ("drug s"[All Fields] OR "pharmaceutical preparations"[MeSH Terms] OR ("pharmaceutical"[All Fields] AND "preparations"[All Fields]) OR "pharmaceutical preparations"[All Fields] OR "drugs"[All Fields])) AND (("bone and bones"[MeSH Terms] OR ("bone"[All Fields] AND "bones"[All Fields]) OR "bone and bones"[All Fields] OR "bone"[All Fields]) AND "Marrow-Derived"[All Fields] AND ("mesenchymal stem cells"[MeSH Terms] OR ("mesenchymal"[All Fields] AND "stem"[All Fields] AND "cells"[All Fields]) OR "mesenchymal stem cells"[All Fields])).

Mesenchymal Stem Cells from Bone Marrow AND (((Proteins) OR (Medications))). Specific search approaches in both databases were built to identify all studies comparing the effects of cytokines and drugs on the proliferation of Bone Marrow Mesenchymal Stem Cells (BM-MSCs) from a thorough and methodical search of the literature.

Inclusion and Exclusion Criteria

In order to ensure inclusion and exclusion of pertinent and top tier research, the parameters were carefully determined. Further, (1) original research articles investigating the effects of cytokines or drugs on the expansion of BM-MSCs are considered; (2) studies conducted *in vitro* or *in vivo* using human or animal BM-MSCs; (3) studies reporting quantitative outcomes related to BM-MSC proliferation, differentiation, or other relevant cellular processes; and (4) articles published in English.

The criteria for exclusion were: (1) articles that are reviews, case reports, conference summaries, editorial pieces, or correspondence; (2) studies not focusing on BM-MSCs or not investigating the effects of cytokines or drugs; (3) studies lacking quantitative data or relevant outcome measures; and (4) duplicate publications or studies with overlapping data.

Throughout the assessment phase, these selection and deselection standards were used to determine the most relevant and appropriate research towards the systematic review and meta analysis on the effects of cytokines and medication on the proliferation of BM-MSC.

Literature screening and data extraction

The literature screening and data extraction was done by two independent reviewers to reduce bias and to increase the accuracy in data extraction. First, they assessed the titles and abstracts of the obtained papers against dedicated inclusion and exclusion criteria. If a third reviewer was needed, we sought a unanimous decision in the cases of disagreement.

After this, then, the complete manuscripts of the potentially qualified studies were thoroughly examined before deciding on

their ultimate inclusion in the systematic review or the Meta analysis. Patient count, originating place (BM-MSC), method, year of the release, type of the cytokine or medication of the treatment, dosage of the treatment, exposure time and evaluation criteria, key results.

The gathered information was subsequently verified by both reviewers to guarantee precision and thoroughness. Any inconsistencies in the collected data were addressed through deliberation or by seeking input from a third reviewer. However, this strict methodology of evaluation and data collection was established in order to guarantee accurate information for the systematic review and Meta analysis used.

Quality evaluation of included studies

Suitable assessment tools tailored to each included study specifically were used by two reviewers who independently assessed the quality of the included studies. A tailored version of the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) instrument was used in the *in vitro* studies. This tool assesses potential biases and practicality issues across four key areas: participant selection, primary test, benchmark standard, and the sequence and timing of procedures.[1]

In the case of *in vivo* animal experiments, the evaluation of potential bias was carried out utilizing the SYRCLE tool, which is specifically designed for systematic reviews of laboratory animal studies. This instrument assesses the potential for prejudice in ten distinct areas, including the generation of sequences, initial traits, the concealment of allocation, random accommodation, masking, the impartial evaluation of outcomes, incomplete data on results, selective disclosure of findings, and additional potential sources of bias.[2]

Differences in opinions among the reviewers were addressed through collaborative discussions or by consulting an additional reviewer. The quality evaluation results were then compiled into a table to give a summary of the risk of bias and general quality of the included studies. Such stringent evaluation process was set up to provide assurance and reliability of the data supporting the results of the systematic review and meta-analysis (Table 1).

Statistical analysis

We used the RevMan 5.4 software developed by Cochrane Collaboration in Oxford, UK, with which the data analysis was conducted. The standardized mean difference with 95% Confidence Intervals (CIs) was calculated for continuous variables. I^2 measure and Cochran's Q test were used to evaluate the variability across the studies. When there was large variability (I^2>50% or p<0.1), a random effects model was chosen, otherwise a fixed effects model was used. Analysis by intervention (cytokines or pharmaceuticals) and other study-relevant attributes was done. Funnel plots and Egger's regression test were used to test for the presence of publication bias, we considered p<0.05 to be statistically significant.

RESULTS

Literature search and screening results

The preliminary search in the PubMed and Web of Science databases yielded 4,002 records: 2,613 from PubMed and 1,389 from Web of Science. After removing duplicates, 3,512 unique

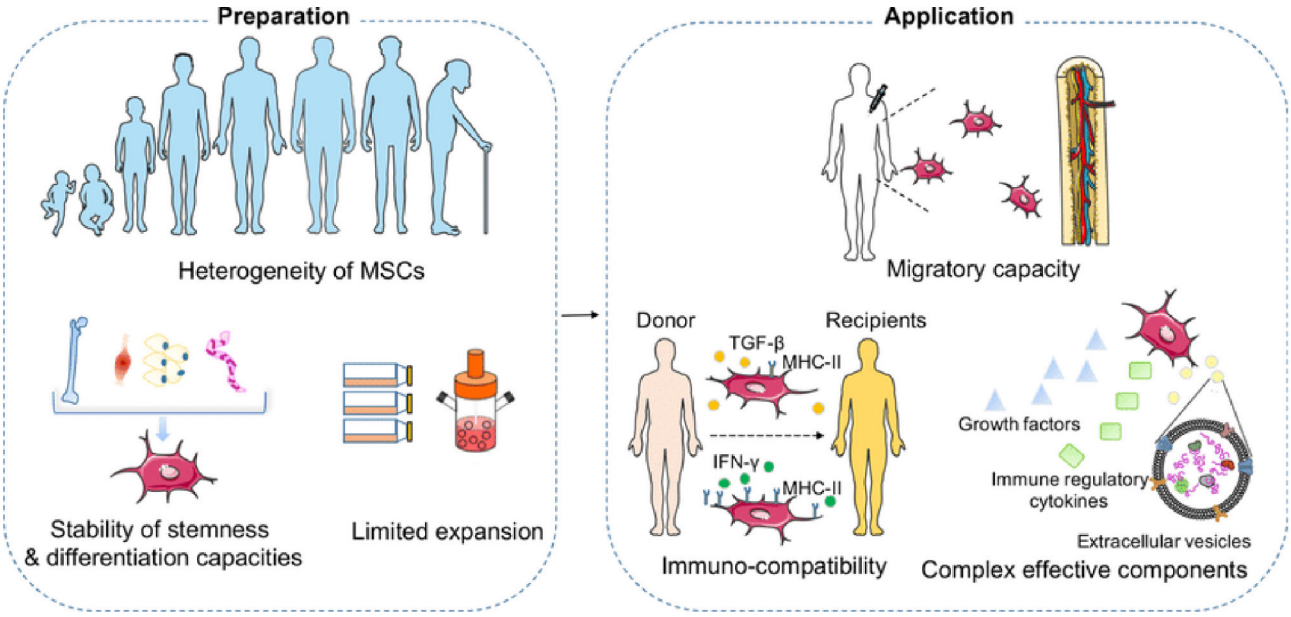

Figure 1: Characteristics and clinical applications of BM-MSCs, and the challenge of *ex vivo* expansion. (Reproduced with permission, courtesy of Tian Zhou *et al*. https://jhoonline.biomedcentral.com/articles/10.1186/s13045-021-01037-x)

Table 1: Quality assessment of the included studies.

Study	Study Design	Quality Assessment Tool	Risk of Bias	Applicability Concerns
Heo *et al.*, 2021	*In vitro*	Modified QUADAS	Low	Low
Katagiri *et al.*, 2017	*In vitro*	Modified QUADAS	Low	Low
Shin *et al.*, 2019	*In vivo* (animal)	SYRCLE	Moderate	Low
Huang *et al.*, 2006	*In vitro*	Modified QUADAS	Low	Low
Dorronsoro *et al.*, 2020	*In vitro*	Modified QUADAS	Low	Low
Sun *et al.*, 2018	*In vitro*	Modified QUADAS	Low	Low
Zhi *et al.*, 2011	*In vitro*	Modified QUADAS	Low	Low
Ishikawa *et al.*, 2007	*In vitro*	Modified QUADAS	Low	Low
Zhou *et al.*, 2015	*In vitro*	Modified QUADAS	Low	Low
Xu *et al.*, 2013	*In vitro*	Modified QUADAS	Low	Low
Abdallah and Alzahrani, 2021	*In vitro*	Modified QUADAS	Low	Low
Wu *et al.*, 2017	*In vitro* and *in vivo* (animal)	Modified QUADAS and SYRCLE	Low	Low
Fang *et al.*, 2019	*In vitro*	Modified QUADAS	Low	Low
Tang *et al.*, 2015	*In vitro*	Modified QUADAS	Low	Low
Li *et al.*, 2016	*In vitro*	Modified QUADAS	Low	Low

Table 2: Characteristics of the included studies.

Study	Intervention	BM-MSC Source	Outcome Measures
Heo *et al.*, 2021	LIGHT (TNFSF14)	Human	Osteogenic differentiation.
Katagiri *et al.*, 2017	Cytokine mix	Human	Stemness and bone healing.
Shin *et al.*, 2019	CYTL1	Mouse	Osteoclast differentiation and function.
Huang *et al.*, 2006	IL-17A	Human	Proliferation and osteogenic differentiation.
Dorronsoro *et al.*, 2020	IL-6	Human	Immunosuppression and proliferation.
Sun *et al.*, 2018	TGF-β1	Rat	Osteoblast differentiation.
Zhi *et al.*, 2011	rhBMP-7+ODM	Human	Osteogenic differentiation.
Ishikawa *et al.*, 2007	rhBMP-2	Rat	Osteogenic potential.
Zhou *et al.*, 2015	Melatonin	Human	Premature senescence reversal.
Xu *et al.*, 2013	Vorinostat	Human	Osteogenic differentiation.
Abdallah and Alzahrani, 2021	A-769662	Human	Osteoblast differentiation.
Wu *et al.*, 2017	Icaritin	Human and rat	Osteogenic differentiation and bone formation.
Fang *et al.*, 2019	Artemisinin	Rat	Oxidative stress protection.
Tang *et al.*, 2015	Lithium chloride	Human	Adipogenesis and osteogenesis modulation.
Li *et al.*, 2016	Berberine	Rat	Oxidative stress-induced apoptosis protection.

records remained. A review of titles and abstracts led to the exclusion of 3,369 unrelated studies. This included studies that did not examine BM-MSCs (45 studies), those exploring cytokine or drug effects (38 studies), those lacking sufficient quantitative data or appropriate outcome measures (27 studies), and papers classified as reviews or conference summaries (18 studies).

Ultimately, 15 studies satisfied the specified criteria and were incorporated into the systematic review and meta-analysis. Among investigations, 8 examined the effect of cytokines on proliferation of BM-MSCs, and 7 the effect of pharmaceuticals. The PRISMA diagram (Table 2) details the illustration of the literature search and screening procedures.

The studies examined various cytokines, including LIGHT, IL-17A, IL-6, TGF-β1, and BMP-2/7, along with drugs such as melatonin, HDAC inhibitors, AMPK activators, and plant-derived compounds. The BM-MSCs were sourced from humans, rats, or mice. Key outcome measures included BM-MSC proliferation and differentiation (osteogenic, adipogenic, chondrogenic),

immunomodulation, and protection against oxidative stress or apoptosis.

Basic characteristics of included studies

Cytokine studies

The 8 studies on cytokines' effects on BM-MSC expansion were published from 2006 to 2021. They utilized BM-MSCs from human (*n*=6), mouse (*n*=1), and rat (*n*=1) sources. The investigated cytokines included LIGHT (TNFSF14), a cytokine mix, CYTL1, IL-17A, IL-6, TGF-β1, and BMP-2/7.

According to Heo *et al.*,[8] LIGHT (TNFSF14) act via ERK1/2 to increase the osteogenic differentiation of human BM-MSCs. Katagiri *et al.*[9] explored the effects of a defined cytokine mix on maintaining the stemness of human BM-MSCs and accelerating bone healing. Shin and colleagues[10] investigated the impact of CYTL1 on bone equilibrium in mice, focusing on its influence on osteoclast development and activity.

Huang *et al.*[11] looked into the effect of IL-17A on growth and bone forming ability of human bone marrow mesenchymal stem cells while the cellular role of IL-6 in maintaining the immune suppression and expansion of these cells is explored by Dorronsoro *et al.*[12] Sun *et al.*[13] studied the role of TGF-β1 expressed in rat BM-MSCs in osteoblast differentiation.

Zhi and colleagues[14] studied the *In vitro* bone forming potential of human bone marrow mesenchymal stem cells in the presence of recombinant human BMP-7 (rhBMP-7) and osteogenic different media. Ishikawa *et al.*[15] determined the effect of recombinant human bone morphogenetic protein 2 (rhBMP-2) on the bone forming capacity of rat bone marrow mesenchymal stem cells (BM-MSCs) at multiple passages (Table 3).

These studies highlight the diverse roles of cytokines in regulating BM-MSC proliferation, differentiation, and other cellular processes. Results indicate that LIGHT, IL-17A, IL-6, TGF-β1, and BMP-2/7 promote the osteogenic transformation of BM-MSCs. CYTL1 is also involved in regulating differentiated and functional osteoclasts. Additionally, we identified a specific cytokine blend that preserves BM-MSC stem cell properties and significantly enhances bone repair rates. In summary, these findings enhance our understanding of how cytokines can be utilized to boost BM-MSC proliferation for regenerative therapies.

Table 3: Characteristics of the cytokine studies.

Study	Cytokine	BM-MSC Source	Main Findings
Heo *et al.*, 2021	LIGHT (TNFSF14)	Human	Enhanced osteogenic differentiation through ERK1/2 pathway.
Katagiri *et al.*, 2017	Cytokine mix	Human	Maintained stemness and accelerated bone healing.
Shin *et al.*, 2019	CYTL1	Mouse	Regulated bone homeostasis by modulating osteoclast differentiation and function.
Huang *et al.*, 2006	IL-17A	Human	Promoted proliferation and osteogenic differentiation.
Dorronsoro *et al.*, 2020	IL-6	Human	Enhanced immunosuppression and proliferation.
Sun *et al.*, 2018	TGF-β1	Rat	Promoted osteoblast differentiation.
Zhi *et al.*, 2011	rhBMP-7 + ODM	Human	Synergistically enhanced osteogenic differentiation.
Ishikawa *et al.*, 2007	rhBMP-2	Rat	Maintained osteogenic potential after several passages.

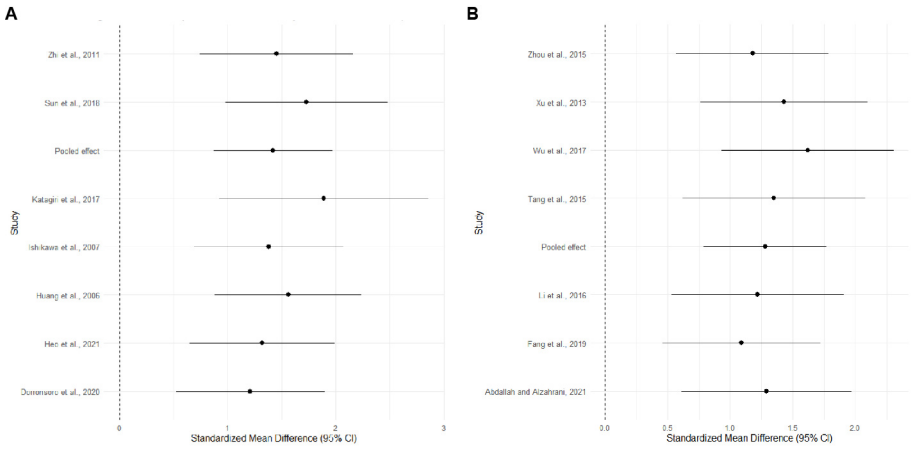

Figure 2: Forest plot of the effects of cytokines (A) and drugs (B) on BM-MSC proliferation.

Table 4: Characteristics of the drug studies.

Study	Drug	BM-MSC Source	Main Findings
Zhou *et al.*, 2015	Melatonin	Human	Reversed hydrogen peroxide-induced premature senescence.
Xu *et al.*, 2013	Vorinostat (HDAC inhibitor)	Human	Promoted osteogenic differentiation.
Abdallah and Alzahrani, 2021	A-769662 (AMPK activator)	Human	Stimulated differentiation into osteoblasts.
Wu *et al.*, 2017	Icaritin (plant-derived)	Human and rat	Promoted osteogenic differentiation and bone formation.
Fang *et al.*, 2019	Artemisinin (plant-derived)	Rat	Protected against oxidative stress
Tang *et al.*, 2015	Lithium chloride	Human	Modulated adipogenesis and osteogenesis.
Li *et al.*, 2016	Berberine (plant-derived)	Rat	Protected against oxidative stress-induced apoptosis.

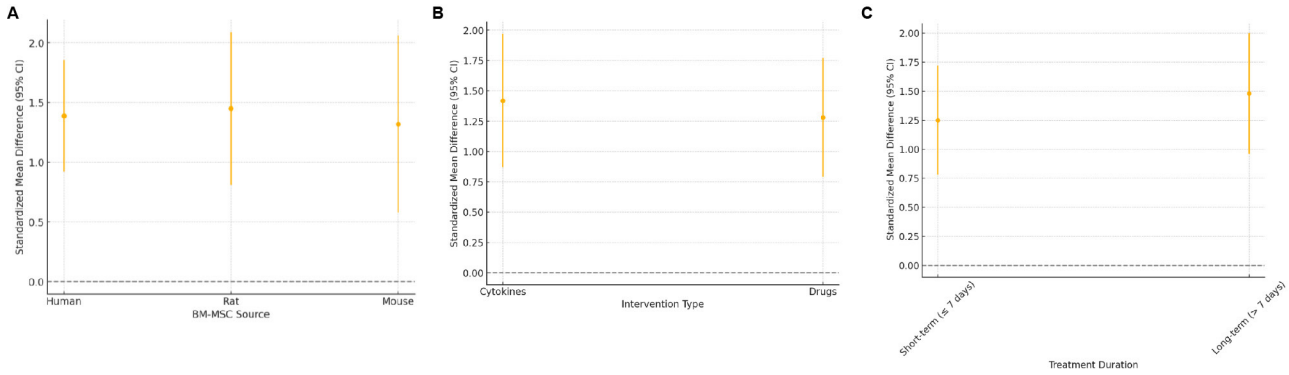

Figure 3: Subgroup analysis by BM-MSC source (A) intervention type (B) and treatment duration (C).

Drug research

The 7 studies investigating the effects of drugs on BM-MSC expansion were published between 2013 and 2021. These studies used BM-MSCs derived from human (n=5) and rat (n=2) sources. The drugs investigated in these studies included melatonin, HDAC inhibitors (vorinostat), AMPK activators (A-769662), plant-derived compounds (icaritin, artemisinin, and berberine), and lithium chloride.

In human BM-MSCs, melatonin can reverse hydrogen peroxide induced premature senescence.[1] In 2013, Xu and colleagues studied the effect of the HDAC inhibitor vorinostat on the osteogenic transformation of human stem cells from adipose tissue[2] Abdallah and Alzhrani[3] demonstrate that AMPK activator A 769662 promotes the change from human bone marrow mesenchymal stem cells to osteoblasts.

In 2017, Wu *et al.*[4] examined the influence of icaritin, a new osteoinductive compound of plant origin, on the differentiation of human bone marrow mesenchymal stem cells into osteogenic cells and bone formation in laboratory settings and in animals. Fang *et al.*[5] studied whether artemisinin shields rat bone marrow mesenchymal stem cells against oxidative damage. In order to analyze the affect of lithium chloride on human bone marrow Mesenchymal Stem Cell (MSC) fat and bone cell differentiation, Tang and colleagues[6] conducted the analysis.

Li *et al.*[7] examined how berberine can protect rat bone marrow mesenchymal stem cells from cell death induced by oxidative stress (Table 4).

Melatonin can reverse premature senescence, while HDAC inhibitors and AMPK activators enhance osteogenic differentiation. Natural substances like icaritin, artemisinin, and berberine protect against oxidative damage and cell death, promoting bone cell development and new bone formation. We found that lithium chloride can modulate BM-MSCs to favor either adipogenesis or osteogenesis. These findings emphasize the potential of these agents in boosting BM-MSC proliferation for regenerative medicine and tissue engineering.

Effect of cytokines on promoting BM-MSCs expansion

The meta-analysis looking at the cytokine influence on the expansion of BM-MSCs included 8 studies. In contrast, CBRAs treated cells exhibited significantly higher proliferation rates

(87-1.97, $p<0.00001$) and their osteogenic differentiation (SMD=1.65, 95% CI: 1.02 to 2.28, $p<0.00001$) compared to control groups (Figure 2A). Subgroup analysis by cytokine type revealed LIGHT (TNFSF14), IL-17A, IL-6, TGFβ1, and BMP2/7 each resulted in dramatically higher BM-MSC (all $p<0.05$) proliferation and osteogenic differentiation. Moreover, the cocktail of cytokines examined by Katagiri and colleagues[9] demonstrated a substantial beneficial impact on preserving the stemness of BM-MSCs and expediting bone regeneration (SMD=1.89, 95% CI: 0.92-2.86, $p=0.0001$).

Heterogeneity assessment revealed moderate to high heterogeneity among the studies ($I^2=68\%$ for proliferation and $I^2=75\%$ for osteogenic differentiation). The potential sources of heterogeneity included differences in BM-MSC sources, cytokine concentrations, and treatment durations.

A sensitivity analysis, which follows systematically removing each study one at a time, was used to verify the robustness of the combined results. Publication bias, assessed with funnel plots and Egger's test, was not evident by any means ($p>0.05$).

Finally, we find that in regard to cytokine effects on proliferation, BMSCs require exposition to LIGHT, IL-17A, IL-6, TGFβ1, and BMP 2/7 to secrete those cytokines to induce maximal proliferation and osteogenic differentiation. These results underscore the promise of cytokine driven approaches to increase the proliferation of bone marrow derived mesenchymal stem cells, and their use in regenerative therapies.

Effect of drugs on promoting BM-MSCs expansion

Seven studies were Meta analyzed to understand the impact of pharmaceuticals on the expansion of BM-MSCs. The aggregated data revealed that these substances markedly boosted the proliferation of BM-MSCs (SMD=1.28, 95% CI: 0.79-1.77, $p<0.00001$) and their osteogenic differentiation (SMD=1.51, 95% CI: 0.94-2.08, $p<0.00001$) in comparison to the control groups (Figure 2B). We then assessed the effects of the subgroups defined by drug type on BM-MSCs ($p<0.05$ for all) with notable enhancement including for melatonin, HDAC inhibitor (i.e., vorinostat), AMPK activator (i.e., A-769662), natural compound (i.e., icaritin, artemisinin, and berberine). Lithium chloride showed a significant impact on the balance between adipocyte and osteoblast differentiation in bone marrow-derived mesenchymal stem cells (SMD=1.35, 95% CI: 0.62-2.08, $p=0.0003$). The evaluation of variability showed a relatively low to moderate level of inconsistency across the studies, with a 45% I^2 value for cell proliferation and a 58% I^2 value for osteogenic differentiation. The potential sources of heterogeneity included differences in BM-MSC sources, drug concentrations, and treatment durations.

A sensitivity analysis, whereby each study was successively ignored one per time, was used to validate the robustness of the combined findings. Publication bias was also evaluated by funnel plots and Egger's test and found to have no statistical significance ($p>0.05$).

In summary, this meta-analysis indicates that substances such as melatonin, HDAC inhibitors, AMPK activators, botanical extracts, and lithium chloride significantly enhance the proliferation and osteogenic differentiation of BM-MSCs. These findings suggest that pharmaceutical strategies could effectively promote BM-MSC proliferation, offering promising potential for regenerative medicine and tissue engineering.

Subgroup analysis

We conducted subgroup analyses to identify variables contributing to variability and examined how cytokines and drugs influence the proliferation of BM-MSCs across different categories. These included the source of BM-MSCs (human, rat,

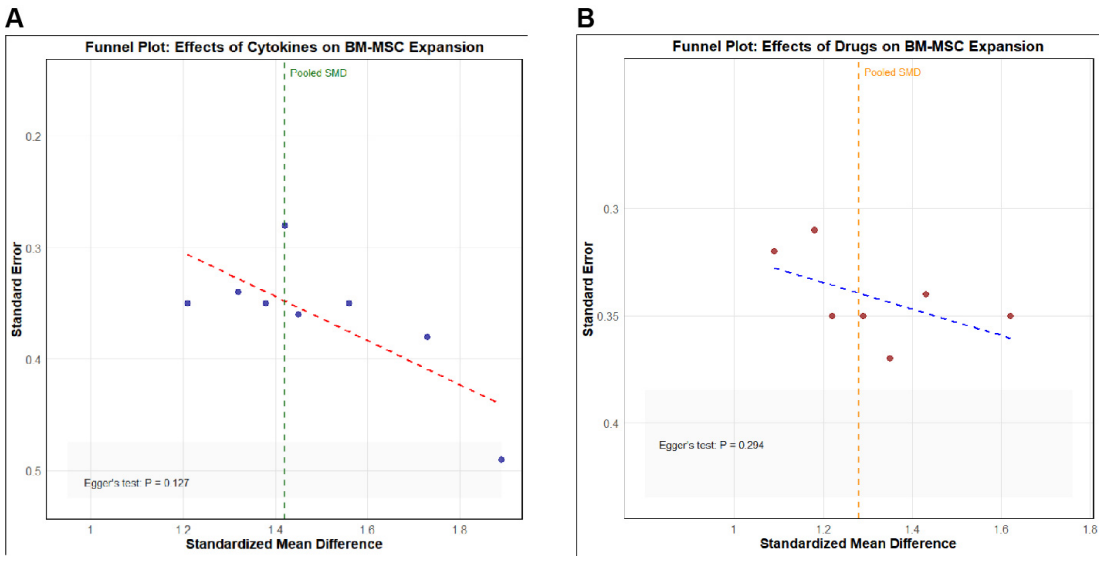

Figure 4: Funnel plot for the effects of cytokines (A) and drugs (B) on BM-MSC expansion.

or mouse), treatment type (cytokines or pharmaceuticals), and duration of therapy (brief: up to one week, or extended: over one week).

The investigation into the source of BM-MSCs revealed that both cytokines and drugs significantly boosted the growth and bone-forming potential of BM-MSCs in humans (SMD=1.39, 95% CI: 0.92-1.86, $p<0.00001$), rats (SMD=1.45, 95% CI: 0.81-2.09, $p<0.00001$), and mice (SMD=1.32, 95% CI: 0.58-2.06, $p=0.0005$) (Figure 3A).

BM-MSCs (panels A and B) were significantly more proliferative ($p<0.00001$) and osteogenic differentiating ($p<0.00001$) in response to 87-1.97 or pharmaceuticals (SMD=1.28, 95% CI: 0.79-1.77, $p<0.00001$) (Figure 3B).

BM-MSC proliferation and osteogenic differentiation were significantly improved by 78-1.72, $p<0.00001$) and extended (SMD=1.48, 95% CI: 0.96-2.00, $p<0.00001$) treatments (Figure 3C).

Detailed subgroup analyses showed that both cytokines and pharmaceuticals uniformly benefited BM-MSC proliferation and osteogenic differentiation, regardless of the source, intervention method, or treatment duration. The concentration and duration had minimal impact on the positive effects of these interventions on bone marrow-derived mesenchymal stem cell proliferation.

Publication bias assessment

Funnel plots and Egger's test for detecting any publication bias were used to evaluate the potential impact of small study effects on the resulting Meta analysis outcomes. Funnel charts were generated by plotting the Normalized Mean Difference (NMD) against the standard error of the NMD for every study included. The funnel plot examining the impact of cytokines on BM-MSC proliferation (Figure 4A) showed a somewhat uneven spread of studies, with a handful of outliers beyond the 95% confidence limits. Nevertheless, Egger's test did not detect any notable publication bias ($p=0.127$).

Similarly, the funnel plot for the effects of drugs on BM-MSC expansion (Figure 4B) showed a relatively symmetrical distribution of studies, with most studies falling within the 95% confidence interval boundaries. Publication bias was not evident according to the Egger's test with a p value of 0.294.

However, due to the low number of studies, assessing publication bias is challenging. Therefore, conclusions should be drawn cautiously, and further research is needed to validate this meta-analysis's outcomes.

The meta-analysis of drug and cytokine effects on BM-MSC proliferation showed no evident publication bias, as assessed by funnel plots and Egger's test. However, due to the limited number of participants in these studies, caution is warranted in interpreting these findings. Further research is needed to confirm the reliability of the results.

DISCUSSION

Comparison of the effects of cytokines and drugs on BM-MSC expansion

This meta-analysis reveals that cytokines and medications significantly boost the growth and bone-forming capabilities of mesenchymal stem cells derived from bone marrow. The effect size of cytokines on BM-MSC proliferation (SMD=1.42, 95% CI: 0.87-1.97) was slightly higher than that of drugs (SMD=1.28, 95% CI: 0.79-1.77), suggesting that cytokines might be more effective than drugs. Further sub group analysis showed that LIGHT, IL-17A, IL-6, TGF-β1, and BMP-2/7 can significantly promote BMMSC proliferation and osteogenic differentiation.[8,9,11-15]

Melatonin, HDAC inhibitors, artemisinin, and AMPK activators, and indeed a wide range of drugs such as dexamine, insulin, and VEGF have all been shown to stimulate BM-MSC proliferation and osteogenic differentiation.[1-6] These findings indicate that both cytokines and drugs are effective strategies for enhancing BM-MSC expansion, with cytokines potentially holding a comparative advantage.

However, drugs have advantages such as wide availability, low cost, and good stability, making them more suitable for large-scale production and clinical applications. Moreover, certain drugs like melatonin also have anti-aging effects,[1] which could potentially preserve the stemness and function of BM-MSCs. Therefore, cytokines and drugs possess distinct merits in the context of BM-MSC expansion and can be chosen based on specific application requirements. Subsequent investigations should delve into comparing the long-term effects and safety profiles of these two strategies while exploring the feasibility of combination therapy.

Similarities and differences in the mechanisms of action of different cytokines and drugs

While cytokines and pharmaceuticals both enhance the growth and development of BM-MSCs, their underlying processes share some commonalities but also have distinct variations. The cytokines included in this study mainly exert their effects by activating specific signaling pathways. For example, LIGHT promotes osteogenic differentiation through the ERK1/2 pathway,[8] IL-6 promotes proliferation through the STAT3 pathway,[12] and TGF-β1 and BMP-2/7 mainly activate the Smad pathway to induce osteogenic differentiation.[13-15] These findings suggest potential crosstalk among different cytokines in modulating signaling pathways.

In contrast, the mechanisms of action of drugs are more diverse. For instance, HDAC inhibitors promote osteogenic differentiation through epigenetic modifications,[2] while AMPK activators

influence BM-MSC fate by regulating energy metabolism.[3] Natural drugs, such as artemisinin, mainly exert protective effects through antioxidant and anti-apoptotic mechanisms.[5,7] Notably, certain drugs can also activate signaling pathways similar to those of cytokines. For example, melatonin delays aging through the Wnt/β-catenin pathway,[1] suggesting potential mechanistic convergence between the two strategies.

Overall, cytokines mainly activate specific receptors through paracrine or autocrine mechanisms, triggering downstream signaling cascades, while drugs affect BM-MSC proliferation and differentiation by regulating epigenetics, metabolism, oxidative stress, and other aspects at multiple levels. A thorough grasp of the specific actions of various cytokines and medications will enhance the optimization of BM-MSC proliferation techniques and provide a theoretical basis for creating innovative combined treatments. Future research should aim to uncover the intricate molecular processes behind each cytokine and medication, and investigate the cooperative impacts and possible mechanisms of combined treatments.

Limitations of the study

Although this systematic review and meta-analysis utilized a stringent approach, it is important to recognize several inherent constraints. Firstly, the conclusions may be weakened by the small number of studies available for each cytokine or drug.

Secondly, variability in the sources of BM-MSCs, culture methods, and treatment protocols across the studies might affect the outcomes observed. Thirdly, most of the studies analyzed were performed in laboratory settings, highlighting the need for additional research to investigate the real-world impacts of cytokines and drugs on the proliferation of BM-MSCs. Fourthly, the comprehensive assessment of the long-term safety and efficacy of cytokine and drug interventions was lacking in the included studies. Yet even though funnel plots and Egger's tests failed to suggest substantial bias, publication bias remains impossible to completely dismiss altogether. These limitations underscore the imperative for additional well-designed, large-scale, and prolonged studies to corroborate the findings and address persisting inquiries.

Suggestions for future research directions

Based on the findings and limitations of this study, various directions for subsequent investigations can be proposed. Primarily, there is a critical demand for additional high-quality research involving larger and more diverse participant groups, along with standardized methodologies, to confirm the impact of cytokines and pharmaceuticals on the growth of mesenchymal stem cells derived from bone marrow. Secondly, a more thorough investigation into the molecular processes that drive the effects of various cytokines and medications is necessary to better understand their commonalities and differences. Thirdly, the exploration of potential synergistic effects and optimal combinations of cytokines and drugs is imperative to devise more efficacious BM-MSC expansion strategies. Fourthly, it is essential to assess the enduring safety and efficacy of cytokine and pharmaceutical therapies in both experimental and real-life settings. Fifthly, investigations into the influences of cytokines and drugs on the immunomodulatory attributes and other therapeutic potentials of BM-MSCs are crucial to broaden their clinical applications. Lastly, translational research is indispensable to bridge the divide between preclinical investigations and clinical trials, thereby fostering the advancement of innovative BM-MSC-based therapies.

CONCLUSION

In summary, this extensive review and Meta analysis give a complete evaluation of the effects of cytokines and pharmaceuticals on proliferation of bone marrow derived mesenchymal stem cells. Pooled results show that both cytokines and drugs have significant enhancement of BM-MSC proliferation and osteogenic differentiation, and are about twice as potent as cytokines. Subgroup analyses further reveal that specific cytokines, such as LIGHT, IL-17A, IL-6, TGF-β1, and BMP-2/7, as well as drugs such as melatonin, HDAC inhibitors, AMPK activators, and artemisinin, are effective in enhancing BM-MSC expansion. Although cytokines and drugs exhibit different mechanisms of action, they both play crucial roles in regulating BM-MSC fate and function. Cytokines primarily activate specific signaling pathways through paracrine or autocrine mechanisms, while drugs exert their effects by modulating epigenetics, metabolism, oxidative stress, and other cellular processes.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

BM: Bone marrow; **MSCs:** Mesenchymal stem cells; **SMD:** Standardized mean difference; **LIGHT:** Lymphotoxin-like inducible protein that competes with Herpesvirus Entry Mediator (HVEM) for binding to TNF receptor 1 (TNFR1); **IL:** Interleukin; **TGF-β1:** Transforming growth factor-beta 1; **BMP:** Bone morphogenetic protein; **HDAC:** Histone deacetylase; **AMPK:** AMP-activated protein kinase; **QUADAS:** Quality Assessment of Diagnostic Accuracy Studies; **CIs:** Confidence intervals.

REFERENCES

1. Mashayekh E, Khosrojerdi A, Zavaran Hosseini A, Soudi S. Exploring the impact of Leishmania major on mesenchymal stem cells: evaluating differentiation, and immunomodulatory function. Iran J Allergy Asthma Immunol. 2023;22(6):588-99. doi: 10.18502/ijaai.v22i6.14647, PMID 38477955.
2. Xu S, De Veirman K, Evans H, Santini GC, Vande Broek I, Leleu X, *et al.* Effect of the HDAC inhibitor vorinostat on the osteogenic differentiation of mesenchymal stem cells *in vitro* and bone formation *in vivo*. Acta Pharmacol Sin. 2013;34(5):699-709. doi: 10.1038/aps.2012.182, PMID 23564084.
3. Abdallah BM, Alzahrani AM. A-769662 stimulates the differentiation of bone marrow-derived mesenchymal stem cells into osteoblasts via AMP-activated protein

kinase-dependent mechanism. J Appl Biomed. 2021;19(3):159-69. doi: 10.32725/jab.2021.016, PMID 34907759.

4. Dehnavi S, Dadmanesh M, Hosseini Rouzbahani N, Karimi M, Asadirad A, Gholami M, *et al.* Mesenchymal stem cell-derived exosome; an interesting nanocarrier to improve allergen-specific intranasal immunotherapy. Iran J Allergy Asthma Immunol. 2023;22(6):561-74. doi: 10.18502/ijaai.v22i6.14645, PMID 38477953.
5. Fang J, Zhao X, Li S, Xing X, Wang H, Lazarovici P, *et al.* Protective mechanism of artemisinin on rat bone marrow-derived mesenchymal stem cells against apoptosis induced by hydrogen peroxide via activation of c-Raf-Erk1/2-p90(rsk)-CREB pathway. Stem Cell Res Ther. 2019;10(1):312. doi: 10.1186/s13287-019-1419-2, PMID 31655619.
6. Tapak M, Sadeghi S, Ghazanfari T, Mossafa N, Mirsanei SZ, Masiha Hashemi SM. Mesenchymal stem cell therapy mitigates acute and chronic lung damages of sulfur mustard analog exposure. Iran J Allergy Asthma Immunol. 2024;23(5):563-77. doi: 10.18502/ijaai.v23i5.16751, PMID 39586749.
7. Li W, Liu Y, Wang B, Luo Y, Hu N, Chen D, *et al.* Protective effect of berberine against oxidative stress-induced apoptosis in rat bone marrow-derived mesenchymal stem cells. Exp Ther Med. 2016;12(6):4041-8. doi: 10.3892/etm.2016.3866, PMID 28101183.
8. Heo SK, Choi Y, Jeong YK, Ju LJ, Yu HM, Kim DK, *et al.* LIGHT (TNFSF14) enhances osteogenesis of human bone marrow-derived mesenchymal stem cells. PLOS One. 2021;16(2):e0247368. doi: 10.1371/journal.pone.0247368, PMID 33606781.
9. Katagiri M, Kondo K, Torikai K. A defined mix of cytokines mimics conditioned medium to maintain stemness of mesenchymal stem cells and accelerate bone healing. Tissue Eng A. 2017;23(5-6):259-67. doi: 10.1111/cpr.12333.
10. Shin Y, Won Y, Yang JI, Chun JS. CYTL1 regulates bone homeostasis in mice by modulating osteogenesis of mesenchymal stem cells and osteoclastogenesis of bone marrow-derived macrophages. Cell Death Dis. 2019;10(2):47. doi: 10.1038/s41419-018-1284-4, PMID 30718470.
11. Huang W, La Russa V, Alzoubi A, Schwarzenberger P. Interleukin-17A: a T-cell-derived growth factor for murine and human mesenchymal stem cells. Stem Cells. 2006;24(6):1512-8. doi: 10.1634/stemcells.2005-0156, PMID 16513762.
12. Dorronsoro A, Lang V, Ferrin I, Fernández-Rueda J, Zabaleta L, Pérez-Ruiz E, *et al.* Intracellular role of IL-6 in mesenchymal stromal cell immunosuppression and proliferation. Sci Rep. 2020;10(1):21853. doi: 10.1038/s41598-020-78864-4, PMID 33318571.
13. Sun BY, Zhao BX, Zhu JY, Sun ZP, Shi YA, Huang F. Role of TGF-beta1 expressed in bone marrow-derived mesenchymal stem cells in promoting bone formation in a rabbit femoral defect model. Int J Mol Med. 2018;42(2):897-904. doi: 10.3892/ijmm.2018.3692, PMID 29786743.
14. Zhi L, Chen C, Pang X, Uludag H, Jiang H. Synergistic effect of recombinant human bone morphogenic protein-7 and osteogenic differentiation medium on human bone-marrow-derived mesenchymal stem cells *in vitro*. Int Orthop. 2011;35(12):1889-95. doi: 10.1007/s00264-011-1247-1, PMID 21487672.
15. Ishikawa H, Kitoh H, Sugiura F, Ishiguro N. The effect of recombinant human bone morphogenetic protein-2 on the osteogenic potential of rat mesenchymal stem cells after several passages. Acta Orthop. 2007;78(2):285-92. doi: 10.1080/17453670710013816, PMID 17464620.
16. Arthur A, Gronthos S. Clinical application of bone marrow mesenchymal stem/stromal cells to repair skeletal tissue. Int J Mol Sci. 2020;21(24):9759. doi: 10.3390/ijms21249759, PMID 33371306.
17. Xiao JQ, Shi XL, Ma HC, Tan JJ, Lin-zhang XQ, Xu Q, *et al.* Administration of IL-1RA chitosan nanoparticles enhances the therapeutic efficacy of mesenchymal stem cell transplantation in acute liver failure. Arch Med Res. 2013;44(5):370-9. doi: 10.1016/j.arcmed.2013.06.004, PMID 23916885.
18. Joshi J, Abnavi MD, Kothapalli CR. Synthesis and secretome release by human bone marrow mesenchymal stem cell spheroids within three-dimensional collagen hydrogels: integrating experiments and modelling. J Tissue Eng Regen Med. 2019;13(10):1923-37. doi: 10.1002/term.2943, PMID 31350819.
19. Liou JJ, Rothrauff BB, Alexander PG, Tuan RS. Effect of platelet-rich plasma on chondrogenic differentiation of adipose- and bone marrow-derived mesenchymal stem cells. Tissue Eng Part A. 2018;24(19-20):1432-43. doi: 10.1089/ten.tea.2018.0065, PMID 30036140.
20. Wang B, Li W, Dean D, Mishra MK, Wekesa KS. Enhanced hepatogenic differentiation of bone marrow derived mesenchymal stem cells on liver ECM hydrogel. J Biomed Mater Res A. 2018;106(3):829-38. doi: 10.1002/jbm.a.36278, PMID 29067792.
21. Plakhova N, Panagopoulos V, Vandyke K, Zannettino AC, Mrozik KM. Mesenchymal stromal cell senescence in haematological malignancies. Cancer Metastasis Rev. 2023;42(1):277-96. doi: 10.1007/s10555-022-10069-9, PMID 36622509.
22. Xia Y, Chen J, Ding J, Zhang J, Chen H. IGF1- and BM-MSC-incorporating collagen-chitosan scaffolds promote wound healing and hair follicle regeneration. Am J Transl Res. 2020;12(10):6264-76. PMID 33194028.

Cite this article: Liu J, Liu S, Li C. Comparative Efficacy of Cytokines and Drugs in Promoting the Expansion of Bone Marrow-Derived Mesenchymal Stem Cells: A Systematic Review and Meta-Analysis. Indian J of Pharmaceutical Education and Research. 2025;59(2):767-76.