# Enhancing mesenchymal stem cell exosomes with traditional herbal therapies for advanced osteoarthritis management

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**Introduction:** Osteoarthritis (OA) is a joint disorder primarily caused by aging and an imbalance in anabolic and catabolic processes [1-2]. Recently, Mesenchymal stem cells (MSCs) have been known to effectively secrete cytokines that support to repair damaged joints, and also diminish inflammation by regulating immune cells, thereby eliminating the progression of OA [3]. However, the direct injection of mesenchymal stem cells is suspected of causing immune rejection, and the exosomes of MSCs are a treatment method that has received more and more attention [4-5]. To enhance the potency of exosomes derived from stem cells, the traditional herbal medicine was pre-treated to strengthen their effectiveness in improving arthritis. In our previous study, we discovered that *Artemisia argyi* water extract (AA) recovered the homeostasis of senescent stem cells [6]. The preliminary results suggest that chondrocytes, which were damaged by ROS, exhibit reduced expression of metalloproteinases and inflammatory genes following the AA-enhanced MSC exosomes. To summarize, traditional herbal medicine AA amplifies MSC exosome production, augmenting their ability to modulate arthritis. Within this study, our objective is to investigate whether Chinese herbal water extracts can enhance MSC exosomes to better regulate the advancement of osteoarthritis.

**Methods:** 1) The exosome extraction is followed by the ExoQuick protocol, which employs a size-restricted polymer to precipitate the exosomes. 2) The size and number of exosomes are assessed using a Zeta-sizer, which utilizes laser light to illuminate the particles and measures their size through dynamic light scattering. 3) Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) is employed to quantify mRNA expression. 4) Western blot is used to determine the protein expression from complete cell lysates. 5) Flow cytometry is assessed to quantify the fluorescence staining in individual cells.

**Results:** The detailed extraction procedure for AA-enhanced MSC exosomes is illustrated in Figure 1A. The AA-enhanced exosomes exhibit increased tetraspanin expression on the cell surface, as depicted in Figure 1B, suggesting a higher concentration of AA-enhanced exosomes in the same volume. This finding is further validated in Figures 1C and 1D, where the size and number of AA-enhanced exosomes are significantly greater than the original. When introduced into damaged chondrocytes (Figure 3A), both types of exosomes demonstrate the ability to restore arthritis-associated metalloproteinases (Figure 2A-B), inflammatory cytokines (Figure 2C-D), and transcriptional factors (Figure 2E-F), indicating their potential to halt the progression of osteoarthritis. The recovery effect is comparable between 40μg/mL of either original or AA-enhanced exosomes (Figure 3B-C). However, the low dose (10μg/mL) of AA-enhanced exosomes exhibits a recovery effect similar to the high dose (40μg/mL) of original exosomes (Figure 3D-E), suggesting that AA-enhanced exosomes possess superior functionality in regulating arthritis metalloproteinases. It was observed that AA enhances the production and function of WJSC exosomes.

**Discussion:** AA-enhanced exosomes have demonstrated clear advantages in terms of both quality and quantity. However, the regulatory influence of AA on WJSC exosomes remains elusive. This study has uncovered an additional dimension by revealing that AA-enhanced exosomes exhibit a larger size compared to their normal counterparts. Investigating how AA treatment leads to the enlargement of WJSC exosomes represents a promising avenue for delving deeper into the mechanisms underlying exosome therapy for arthritis. Importantly, this study marks the pioneering use of Chinese herbal water extract to augment MSC exosome function. In the future, the exploration of various herbal water extracts in combination could serve as a promising new platform for discovering optimal exosome enhancements.

**Significance:** This study introduces a novel perspective in which Chinese herbal water extracts have the potential to boost the production and functionality of MSC exosomes. It also represents the first investigation into the impact of Chinese herbal water extracts on exosomes. The potential for combining various Chinese herbal therapies in the future holds promise.

**References:** 1. Martel-Pelletier J, et al. Osteoarthritis. *Nat Rev Dis Primers.* 2016;2:16072. 2. Nedunchezhiyan U, et al. Obesity, Inflammation, and Immune System in Osteoarthritis. *Front Immunol.* 2022;13:907750. 3. Jiang Y. Osteoarthritis year in review 2021: biology. *Osteoarthritis Cartilage.* 2022;30(2):207-215. 4. Yu H, et al. Research progress in the use of mesenchymal stem cells and their derived exosomes in the treatment of osteoarthritis. *Ageing Res Rev.* 2022;80:101684. 5. Kim YG, et al. Mesenchymal Stem Cell-Derived Exosomes for Effective Cartilage Tissue Repair and Treatment of Osteoarthritis. *Biotechnol J.* 2020;15(12):e2000082. 6. Ho TJ, et al. Artemisia argyi exhibits anti-aging effects through decreasing the senescence in aging stem cells. *Aging (Albany NY).* 2022;14(15):6187-6201.

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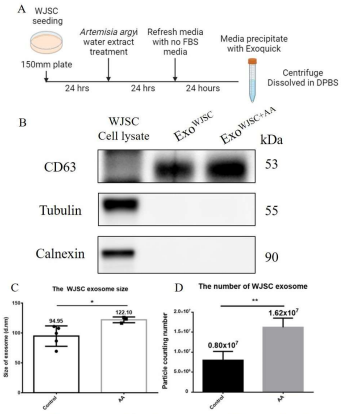


Figure 1. The characteristic difference between original and AA-enhanced WJSC exosome. (A) The scheme of AA-enhanced WJSC exosome extraction. (B) The protein expression of original and enhanced exosomes (C-D) The size distribution and number of exosomes estimated by Zeta-sizer. * indicates p<0.05. ** indicates p<0.01. n=3 independent experiments.

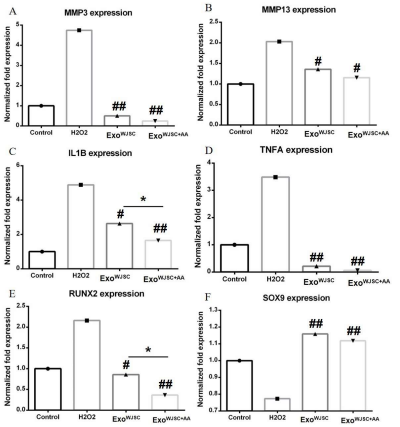


Figure 2. The enhanced recovery effect on gene expression. (A-B) The gene expression of arthritis katabolic enzymes metalloproteinase 3 and 13. (C-D) The inflammatory associated genes *IL1B* and *TNFA*. (E-F) The expression of arthritis associated transcriptional factors. * indicates p<0.05. Comparing to H2O2 # indicates p<0.05. ## indicates p<0.01. n=3 independent experiments.

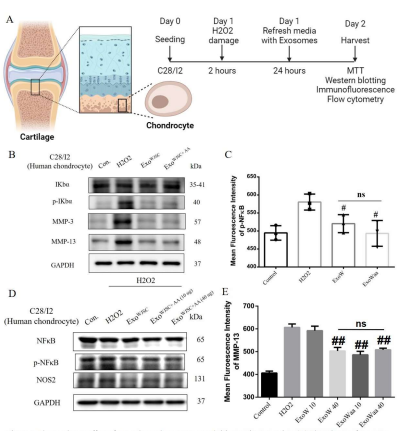


Figure 3. The regulatory effect of AA-enhanced exosome on arthritis protein expression. (A) The scheme of exosome treatment (B-C) The recovery effect from 40μg/mL of exosome treatment on ROS damaged human chondrocytes by Western blot and Flow cytometry (D-F) The better arthritis effect in low dose of AA-enhanced exosomes by Western blot and Flow cytometry. Comparing to H2O2 # indicates p<0.05. ## indicates p<0.01. n=3 independent experiments.