

Therapeutic Effects of Exosomes in Animal Models of Pulmonary Hypertension: A Systematic Review

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ADMINISTRATIVE INFORMATION

Support - Universiti Kebangsaan Malaysia.

Review Stage at time of this submission - Preliminary searches.

Conflicts of interest - None declared.

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Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 13 November 2025 and was last updated on 13 November 2025.

INTRODUCTION

Review question / Objective To systematically evaluate the therapeutic effects and underlying mechanisms of exosome-based interventions in animal models of pulmonary hypertension (PH).

Condition being studied PH, a progressive cardiopulmonary disorder characterized by elevated pulmonary arterial pressure, vascular remodeling, and right ventricular hypertrophy, which ultimately leads to right heart failure.

METHODS

Search strategy This systematic review will follow the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) guidelines and the Population, Intervention, Comparison, Outcome, and Study (PICOS) framework. To identify relevant studies on the role of exosomes in PH, a comprehensive search will be conducted across three major databases: Ovid

MEDLINE, Scopus, and Web of Science. The search strategy will include keywords and their combinations such as: (“pulmonary hypertension” OR “pulmonary vascular disease” OR “elevated pulmonary artery pressure” OR “pulmonary arterial hypertension” OR “PAH”) AND (“exosome*” OR “extracellular vesicle*” OR “EV” OR “microvesicle*” OR “ectosome*” OR “secretome*” OR “nanovesicle*”) AND (“rodent*” OR “rat*” OR “mouse” OR “mice” OR “in vivo” OR “preclinical”). The titles and abstracts of retrieved studies will be screened for relevance based on predefined inclusion and exclusion criteria aligned with the PICOS framework, focusing specifically on exosome-based treatments in animal models of PH. There will be no geographical or publication date restrictions, and studies published in any language will be considered. Additionally, reference lists of included articles will be manually screened to identify any potentially relevant studies not captured in the initial database search.

Participant or population Animal studies: This review will include preclinical in vivo animal models

of PH. Eligible studies must clearly describe the experimental method used to induce PH, such as chronic hypoxia exposure, monocrotaline (MCT) injection, or SU5416/hypoxia (SuHx) models. All animal species (e.g., rats, mice) and both sexes will be considered, regardless of age or weight.

Exclusion: Studies involving human participants, other forms of hypertension (e.g., essential, systemic, secondary, or gestational hypertension), or animals with comorbid conditions unrelated to pulmonary hypertension will be excluded. In addition, *in vitro*, *ex vivo*, clinical trials, and any non-animal experimental models will not be considered.

Intervention Studies that use exosomes as the main treatment will be included, regardless of their source, isolation method, or route of administration. Exosomes may originate from mesenchymal stem cells, cardiac progenitor cells, endothelial cells, or other relevant sources. Included studies must describe how the exosomes were prepared, characterized, and administered, including details on dosage, frequency, and treatment duration.

Exclusion: Studies that use other treatments instead of exosomes, or that combine exosomes with drugs, compounds, or gene therapies, will be excluded. Studies using modified or engineered exosomes or those delivering exosomes to unrelated organs will also be excluded.

Comparator The comparator group in eligible studies must include a control or reference group to assess the therapeutic effects of exosomes. Acceptable comparator groups include:

- Untreated PH animals,
- Healthy control animals,
- Placebo- or vehicle-treated groups, and
- Standard therapy groups, such as those treated with sildenafil or other conventional pulmonary vasodilators.

Exclusion: Studies without a defined control or comparator group will be excluded from this review.

Study designs to be included Only preclinical *in vivo* animal studies investigating the therapeutic effects of exosomes in PH will be included. These may involve experimental models using rodents or other animals, regardless of species, age, weight, or sex. **Exclusion:** Studies using *in vitro* cell cultures, *ex vivo*, clinical trials, or other non-animal study designs will be excluded. Additionally, reviews, editorials, conference abstracts, book/book chapters, case reports, and guidelines will not be included.

Eligibility criteria This review will include all preclinical *in vivo* studies investigating the therapeutic effects of exosomes in animal models of PH. Studies must report at least one relevant hemodynamic, histological, or molecular outcome related to PH progression or recovery. The source of exosomes, administration route, and treatment parameters must be clearly described.

Information sources A comprehensive literature search will be conducted using three major electronic databases: Ovid MEDLINE, Scopus, and Web of Science. The primary search terms will include “pulmonary hypertension” OR “pulmonary vascular disease” OR “elevated pulmonary artery pressure” OR “pulmonary arterial hypertension” OR “PAH” AND “exosome*” OR “extracellular vesicle*” OR “EV” OR “microvesicle*” OR “ectosome*” OR “secretome*” OR “nanovesicle*” AND “rodent*” OR “rat*” OR “mouse” OR “mice” OR “*in vivo*” OR “preclinical”.

Main outcome(s) The primary outcomes of this review will focus on hemodynamic improvements and functional changes following exosome treatment in animal models of PH. These include:

- Mean pulmonary artery pressure (mPAP)
- Pulmonary vascular resistance (PVR)
- Right ventricular systolic pressure (RVSP)
- Pulmonary ejection time

Where available, these parameters will be extracted as mean values $\pm$ standard deviation (SD) or as percentage changes compared with control groups.

Additional outcome(s) Additional outcomes will include changes in lung and heart structure, such as reduced vascular remodeling and right ventricular hypertrophy (RVH). Improvements in arterial blood gas (ABG) and oxygen levels will also be considered. Molecular outcomes will include changes in inflammatory, fibrotic, and oxidative stress markers, as well as the microRNA or protein contents of exosomes and their related signaling pathways, if available.

Data management Article screening: Five reviewers (N.A.M., S.K., L.R.E., A.K. and M.Z.H.B) will independently screen the titles and/or abstracts of exported studies against the predetermined inclusion and exclusion criteria. Upon completion of the initial screening, the full-text versions of eligible studies will be obtained for further assessment. Any disagreement will be resolved through discussion with a sixth reviewer (A.U.). A PRISMA flow diagram will be utilized to report the study selection process.

Data extraction:

Data from all included studies will be extracted using a standardized data collection form by five independent reviewers (N.A.M., S.K., L.R.E., A.K. and M.Z.H.B). Any disagreement will be resolved through discussion with a sixth reviewer (A.U.). All extracted data will be compiled using Microsoft Excel. Any missing data will be requested from the corresponding authors via email.

The information collected will include:

- Study details: author name, publication year, country, and study design.
- Animal model characteristics: species, sex, weight, age, method used to induce PH, and sample size.
- Intervention details: source of exosomes, isolation method, dose, route, and timing of administration.
- Comparator: type of control or reference group used.
- Outcomes of interest as described in the PICOS framework.

Quality assessment / Risk of bias analysis The risk of bias (RoB) for each included study will be assessed independently by five reviewers (N.A.M., S.K., L.R.E., A.K. and M.Z.H.B). Any disagreement will be resolved through discussion with a sixth reviewer (A.U.). For animal studies, the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) RoB tool will be used.

This tool evaluates key domains such as:

- Selection bias: randomization and baseline comparability,
- Performance bias: blinding of investigators and random housing,
- Detection bias: blinding of outcome assessment,
- Attrition bias: completeness of outcome data, and
- Reporting bias: selective outcome reporting.
- Other bias

Each domain will be rated as low, high, or unclear. The overall results will be summarized in tables and described narratively. The RoB assessment will not be used as an exclusion criterion.

Strategy of data synthesis Study characteristics and outcome data will be tabulated and described narratively. A meta-analysis using the fixed-effect or random-effects model will be performed, where appropriate, using the Review Manager (RevMan) Web software. All the meta-analysis results will be visually presented as forest plots. When ten or more studies are available, a random-effects meta-regression will be performed to further investigate the potential sources of heterogeneity.

Subgroup analysis If sufficient data are available, subgroup analyses will be performed to explore factors that may influence the therapeutic effects of exosomes in PH. The following variables will be considered:

- Animal variations (e.g., species, sex, age, and weight)
- Source of exosomes (e.g., mesenchymal stem cells, endothelial cells, or cardiac progenitor cells)
- Method of pulmonary hypertension induction (e.g., MCT, hypoxia, or SuHx models)
- Route and timing of administration (e.g., intravenous, intratracheal, or direct pulmonary delivery)
- Dosage and treatment duration
- Exosomal contents (e.g., specific microRNAs or proteins involved in the therapeutic mechanisms)

These subgroup analyses will help determine how different experimental conditions and exosome characteristics affect treatment outcomes in PH models.

Sensitivity analysis A sensitivity analysis will only be conducted if a meta-analysis is performed. Sensitivity analysis will be performed by systematically including or excluding studies to assess the robustness of the overall results.

Language restriction None.

Country(ies) involved Malaysia.

Keywords Exosomes; Extracellular vesicles; Preclinical studies; Pulmonary hypertension; Right ventricular hypertrophy; Vascular remodeling.

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