Dose-related Effects of Resveratrol in Different Models of Pulmonary Arterial Hypertension: A Systematic Review

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Abstract: Background: Pulmonary Arterial Hypertension (PAH) is a severe and progressive disease of pulmonary arterioles. This pathology is characterized by elevation of the pulmonary vascular resistance and pulmonary arterial pressure, leading to right heart failure and death. Studies have demonstrated that resveratrol possesses a protective effect on the mechanisms related to the genesis of the PAH induced by different models.

Objective: This study aimed to investigate the dose-related effects of resveratrol in different models of pulmonary arterial hypertension.

Methods: To identify eligible papers, we performed a systematic literature search on Scielo, PubMed, and Scholar Google. The research was limited to articles written in English in the last 10 years. We used the following descriptors to search: Pulmonary Arterial Hypertension and Resveratrol, OR Resveratrol, and Animal models of Pulmonary Arterial Hypertension, OR Resveratrol, and in vitro models of Pulmonary Arterial Hypertension.

Results: 1724 studies were identified through the descriptors used, fifty-five studies with different models of pulmonary arterial hypertension were selected for the full review, forty-four were excluded after application of exclusion and inclusion criteria, totaling eleven studies included in this systematic review.

Conclusion: The results showed that resveratrol, at low and high doses, protects in a dose-dependent manner against the development of PAH induced through monocrotaline, normoxia and hypoxia models. In addition to having chemopreventive, anti-inflammatory, antioxidant and anti-proliferative properties. In the case of PAH-related myocardial injury, resveratrol protects cells from apoptosis, thus working as an antiapoptotic agent.

Keywords: Pulmonary hypertension, resveratrol, drug therapy, administration and dosage, inflammation, oxidative stress, right ventricular failure.

1. INTRODUCTION

Pulmonary Arterial Hypertension (PAH) is a progressive disease with hemodynamic consequences characterized by an increase in pulmonary vascular resistance, leading to right ventricular overload and right ventricular failure [1-3]. It is a pathological condition common to a variety of etiologies; however, its pathophysiological mechanisms are not well known [4-6].

The average pulmonary artery pressure is 14±3 mmHg [7], PAH is defined as (mPAP) equal to or exceeding 25 mmHg according to An official American Thoracic Society clinical practice guideline: diagnosis, risk stratification, and management of pulmonary hypertension of sickle cell disease [8]. It can also be observed that the right ventricular function is compromised when there is an acute increase in the pressure in the pulmonary artery with levels greater than 40 mmHg. However, when presented chronically, it can determine the adaptations of right ventricular function up to levels close to 60 mmHg without impairment of function [9-11].
Epidemiological data of this disease differ in the literature. However, it is possible to highlight values of 15-26 cases per million inhabitants per year in the world, affecting predominantly women with a female to male ratio of 1.7:1; and the onset of symptoms mainly after the second or third decade of life [12, 13]. Highlight also a high prevalence in collagen diseases, with 15% of individuals presenting PAH frequently associated with systemic sclerosis and in the masonic schistosomiasis responsible for one-third of the cases of PAH in Brazil, more commonly reported in patients with the hepatosplenic form [14-17].

PAH has been hemodynamically classified as pre-capillary, post-capillary, or combined pre- and post-capillary since there may be overlaps among these conditions [18]. The first is defined by the concomitant presence of mPAP >20 mmHg, pulmonary arterial wedge pressure (PAWP) ≤15 mmHg, and pulmonary vascular resistance (PVR) ≥3 Wood Units (WU). This group comprises the majority of the etiopathogenic factors of the PAH [19]. The second group is characterized when the pulmonary capillary pressure exceeds 15 mmHg with preserved ejection fraction. This category includes left heart diseases, systolic, or diastolic left ventricular dysfunction, which can lead to heart failure [20]. This latter group may present with isolated post-capillary PAH or combined post-capillary PAH with a pre-capillary component, as indicated by an elevated diastolic pressure gradient and an increased PVR [18]. Because of this, alterations can be observed between the different forms of PAH such as vascular remodeling, including the cellular proliferation of the intima and media, endothelial dysfunction, increased vasoconstriction and activation of inflammatory processes [21-26].

Some mechanisms of the pathophysiology of the PAH are still not fully elucidated. The current therapies used to treat this disease include the use of drugs that act, promoting a decrease in pulmonary artery resistance and inducing vasodilatation. The three different pathways used are selective endothelin receptor antagonist, nitric oxide pathway as a selective inhibitor of phosphodiesterase type 5 and guanylate cyclase stimulant, and prostacyclin pathway by the adenosine monophosphate [21, 27-29].

Like this, the use of in vivo and in vitro models that replicate the developmental processes of this pathology and allow the evaluation of new substances in the treatment of PAH have been performed. Among these substances is the resveratrol, a polyphenol that acts with antioxidant and anti-inflammatory properties, modulator of endothelial function, inhibitor of vascular smooth muscle cell proliferation [30-32], and regulator of the expression of factors responsible for activation, induction or even in the control of genes involved in the vasoconstriction [9, 12, 33].

Thus, this review article aimed to investigate the effects related to the dose of resveratrol in different models of pulmonary arterial hypertension.

2. METHODS

2.1. Systematic Review Strategies

A systematic review has been carried out to summarize the available results of experimental studies. To identify eligible papers, we performed a systematic literature search on Scielo, Pubmed, and Scholar Google. The research was limited to articles written in English in the last 10 years; thus, papers published between April 2009 and April 2019 were included. We use the following descriptors to search: Pulmonary Arterial Hypertension and Resveratrol, OR Resveratrol, and animal models of Pulmonary Arterial Hypertension, OR Resveratrol, and in vitro models of Pulmonary Arterial Hypertension. Besides, scanning of reference lists was performed from the retrieved studies to identify any articles that may have been missed from the literature search. The studies included in this review were selected based on the inclusion and exclusion criteria presented in Fig. (1).
2.2. Study Selection Criteria

To select only the relevant studies, the titles and abstracts of all the citations identified by the bibliographic research were examined. For this purpose, all those studies were included that investigated the specific topic of resveratrol use as primary therapy and aspects related to pulmonary arterial hypertension in studies that involved in vivo and in vitro models.

For characterization aspects of included studies, the following criteria were adopted as the most important: species, strain, sex or cell culture, age, weight, number of control and treated animals, Pulmonary Arterial Hypertension model, Resveratrol dose, drug administration, follow-up in weeks and aim of the study. Then all the data needed for the characterization were organized and presented in tabular form. The data that were not identified in the studies were filled out with n.e (not evaluated).

3. RESULTS

Of the 1724 studies identified through a systematic review of the literature, fifty-five studies with different models of pulmonary arterial hypertension were identified for the full considerations. Eight duplicate articles were excluded, five were not in English, nine were not Pulmonary Arterial Hypertension model, eleven with no use of Resveratrol or not used as primary therapy, and eleven studies did not have the outcome of interest. Total eleven studies were included and characterized as shown in Table 1. The main results of the studies included in this review are evidenced in Table 2.

Table 1. Characteristics of studies included in the systematic review.

| Author    | Species, Strain, Sex or Cell Culture | Age (Weight) | No. of Control Animals | No. of Treated Animals | PAH Model | Resveratrol Dose | Drug Administ. | Timing of Resveratrol Therapy | Followup (Weeks) | Aim                                           |
|-----------|-------------------------------------|--------------|------------------------|------------------------|-----------|------------------|----------------|-----------------------------|-----------------|-----------------------------------------------|
| Csiszar et al. [15](2009) | Rats, Sprague-Dawley, Males. | Adults (300g). | Control: 14 or 21 days; MCT: 14 or 21 days; (n=6, per group). | MCT + RES: 14 and 21 days (n=6, per group). | Monocrotaline induced PAH (60 mg/kg SC). | 25 mg/kg per day | In drinking water. | From day 1 to day 14 or day 21 after MCT injection. | Up to 3 weeks | To investigate the efficacy of resveratrol to present PAH. |
| Yang et al. [25](2010) | Rats, Sprague-Dawley, Males. | 10 weeks old (180-200g). | Control (n=8); MCT (n=12). | MCT + RES: 10 mg/kg MCT + RES: 30 mg/kg (n=8, per group). | Monocrotaline induced PAH (50 mg/kg SC). | 10 or 30 mg/kg per day | Intragastric | 1 to day 21 after MCT injection. | 3 weeks | To investigate the protective effects of resveratrol against right ventricular hypertrophy. |
| Patelli Lucas and Campelo [25](2012) | Rats, Sprague-Dawley, Males. | 8-10 weeks old (>300g). | Control; Saline or resveratrol; MCT saline or resveratrol (n=8-12, per group) | MCT + RES: MCT + Sildenafil; (n=8-12, per group). | Monocrotaline induced PAH (50 mg/kg IP). | 3 mg/kg per day | In drinking water. | From day 28 to day 42 after MCT injection. | 6 weeks | To verify the atrophy pathways in the pulmonary artery after the use of resveratrol. |
| Patelli et al. [31](2012) | Rats, Sprague-Dawley, Males. | 8-10 weeks old (approximately 300g). | Control; MCT; (n=12, per group). | Saline + RES; MCT + RES; (n=12). | Monocrotaline induced PAH (50 mg/kg IP). | 3 mg/kg per day | In drinking water. | From day 28 to day 42 after MCT injection. | 6 weeks | To assess longitudinal changes in cardiac function, morphology, and perfusion. |
| Chen et al. [30](2014) | In vivo: Rats, Sprague-Dawley, Males. | Neonatal 2 days old (n.e). | Vehicle: normoxia or hypoxia; (n=8-12 per group). | Resveratrol: normoxia or hypoxia; (n=8-12 per group). | In vivo: Hypobaric hypoxic chamber with barometric pressure maintained at 435 – 5 Torr for 14 days. | From day 1 to day 14 after placed into hypoxic chamber. | 2 weeks | To investigate the effects of the present hypoxia-induced proliferation in human pulmonary artery smooth muscle cells via inhibition of arginase II induction. |
| Chen et al. [30](2014) | In vitro: Human PASMC. | Group 0 – Positive control. | Group 40 µM Group 80 µM Group 100 µM, in 21% or 1% of O2. | In vitro: 100 mg/kg per day. | In vitro: 40, 80, or 100 µM. | 30 min, 24, 48, or 120 hours. | 1 week | In vitro | To investigate the effects of the present hypoxia-induced proliferation in human pulmonary artery smooth muscle cells via inhibition of arginase II induction. |
| Author (Year) | Species, Strain, Sex or Cell Culture | Age (Weight) | No. of Control Animals | No. of Treated Animals | PAH Model | Resveratrol Dose | Drug Administ. | Timing of Resveratrol Therapy | Followup (Weeks) | Aim |
|---------------|------------------------------------|-------------|-----------------------|-----------------------|-----------|-----------------|----------------|-----------------------------|----------------|-----|
| Zhou et al. [41] (2015) | Rats, Sprague-Dawley, Males. | Adults (280-300g) | Control; MCT + vehicle; (n=20, per group). | MCT + RES 2.5 mg/kg; MCT + RES 20 mg/kg; (n=20, per group). | Monocrotaline-induced PAH (60 mg/kg SC). | 2.5 or 20 mg/kg per day | Intra gastric | From day 1 to day 14 or day 21 after MCT injection. | 3 weeks | To examine the effects of resveratrol on cardiac and pulmonary trunk remodeling, and common plasma markers of vascular function. |
| Whitaker et al. [26] (2016) | Rats, Sprague-Dawley, Males. | Adults (260±6g). | Control; MCT; (n=12, per group). | MCT + RES (n=12, per group). | Monocrotaline-induced PAH (60 mg/kg SC). | 25 mg/kg per day | Intra gastric | From day 1 to day 21 after MCT injection. | 3 weeks | To examine the effects of resveratrol on cardiac and pulmonary trunk remodeling, and common plasma markers of vascular function. |
| Xue et al. [19] (2016) | Rats, Sprague-Dawley, Males | Adults (n.e) | Normoxia; Hypoxia; (n=6, per group). | Normoxia + RES; Hypoxia + RES; (n=6, per group). | Normoxia and Hypoxia-induced PAH per 28 days (Normoxia: ambient barometric pressure: 718 mmHg/PO2, 150.6 mmHg; Hypoxia: 380 mmHg/PO2, 79.6 mmHg). | 40 mg/kg per day | Intra gastric | From day 1 to day 21 after placed into normoxia and hypoxic chamber. | 3 weeks | To investigate the effects of resveratrol on HPH development. |
| Guan et al. [39] (2017) | Rats, Sprague-Dawley, Males | 50 days old (150-180g) | Positive control (cells + saline + hypoxia). | RES (10, 30 or 100 µmol/l); Resveratrol + LY-294002; Resveratrol + PI3K inhibitor; Resveratrol + IGF-1; Evaluated in 24, 48 and 72 hours. | In vitro: A hypoxic environment was induced using an autonomous plexiglass chamber supplied with 5% CO2 and 95% N2 at 20 ml/min. | Resveratrol (10, 30 and 100 µmol/l). | In vitro | 24, 48 and 72 hours. | 1 week | To investigate the role of resveratrol by examining alterations in expression levels of genes associated with the PI3K/AKT pathways, proliferation, and migration. |
| Yue et al. [31] (2017) | Rats, Wistar, Males. | Adults (200–300g) | Normoxia (n= n.e) | Hypoxia (n= n.e). | In vivo: Hypoxia-induced PAH per 21 days (hypoxia involved fractional inspired oxygen of 0.21 and 0.12). | 25 mg/kg per day | Intra gastric | In vivo: From day 1 to day 21 after placed into hypoxic chamber. | 3 weeks | To investigate the action and potential mechanism of resveratrol on PAH, focusing on the role of SIRT1 (Silent information Regulator 1) in apoptosis of pulmonary artery smooth muscle cells. |
| Shi et al. [28] (2018) | Rats, Sprague-Dawley, Males. | Adults (190–200g) | Control; MCT; (n=8, per group). | MCT and PF543; MCT and PDTC; MCT, PF543 and PDTC; MCT and resveratrol; MCT, resveratrol, and PDTC; (n=8, per group). | MCT- induced PAH (60 mg/kg IP). | 25 mg/kg per day | Intra gastric | From day 1 to day 28 after MCT injection. | 4 weeks | To explore the molecular mechanisms underlying SphK1 inducing pulmonary vascular remodeling and RES suppressing PAH. |

Note: No: number; PAH: pulmonary arterial hypertension; MCT: monocrotaline; RES: resveratrol; SC: subcutaneous; IP: intraperitoneal; n.e: not evaluated; HPH: hypoxia induced pulmonary hypertension; LY-294002: (2-(4-ethylphenyl)-3-phenyl-4H-1-benzopyran-4-one), an inhibitor of phosphoinositide-3 kinase; PIK; phosphoinositide 3-kinase; IGF-1: insulin-like growth factor 1; AKT: Protein kinase B, SRT1720: activating drug of SIRT1; PF543: the drug that acts as a specific inhibitor of sphingosine kinase 1; PDTC: Pyrroolidine Dithiocarbamate, an anti-oxidant; SphK1: sphingosine kinase 1;
Table 2. Detailed information on effects of the resveratrol in vitro and in vivo models of pulmonary arterial hypertension.

| Author (year) | Cardiovascular Function | Endothelial Function | Inflammatory Markers | Oxidative Markers | Others Variables |
|---------------|-------------------------|----------------------|----------------------|-------------------|------------------|
| Casas et al. [35] (2009) | Resveratrol treatment from day 1 normalized right ventricular systolic pressure in MCT-injected rats at both the 2- and 3-week periods; Resveratrol treatment prevented MCT-induced RV hypertrophy; Resveratrol treatment normalized medial wall thickness, avoiding the increase in PASMC mass in vessels of MCT-treated rats; The number of PCNA-positive cells was considerably reduced in animals treated with resveratrol. | Increase of small pulmonary arteries relaxation; Improved eNOS expression. | Resveratrol treatment significantly attenuated the mRNA expression of IL-6, IL-1, TNF-α, PDGF β, TGF-β, MCP-1. | Expression of NAD(P)H oxidase subunits were upregulated; Downregulation of NOX-1 and gp91phox gene; Improved eNOS expression. | n.e |
| Yang et al. [29] (2010) | The dose of 30mg/kg showed lower right ventricular hypertrophy, right ventricle mass index, cardiomyocyte length, and cardiomyocyte cross-sectional area among the MCT-induced PAH groups; RV free wall thickness was decreased by 25.26 and 40.67%, in the dose of 10 and 30mg/kg respectively, compared with untreated MCT group; Pulmonary arterial acceleration time showed a significant increase (50.63%) and 67.75%, respectively, in the 10 and 30 mg/kg in the resveratrol treated MCT groups; Treatment with 10 and 30 mg/kg decreased right ventricular systolic pressure by 26.26% and 37.36%, respectively. There were no significant changes in mean systemic blood pressure among the groups. | n.e | n.e | After treatment with resveratrol (10 and 30 mg/kg), the morphological structure of the myocardium exhibited less hypertrophy, without fibrosis, and the myofilibrillae were lined up; Treatment with resveratrol (10 and 30 mg/kg) significantly improved mitochondrial proliferation, swelling, vacuolization, and medullary sheet-like degeneration, also decreasing sarcoplasmic reticulum and dissolution of the myofibrillres, observing still decrease of broken Z-lines and irregular pattern of transverse striaetions. | n.e |
| Pattani, Lun, and Garnep [25] (2011) | Resveratrol and Sildenafil (used as a clinical reference standard) treatments initiated 28 days after MCT injection caused a significant reduction in right ventricular systolic pressure in MCT-injected rats, with no effect in saline controls; Right ventricular hypertrophy, measured by the RV/LV+S, show decrease only in the resveratrol therapy group; There was no decrease in right ventricular hypertrophy after treatment with resveratrol. | Resveratrol restores acetylcholine-induced relaxation and KCI-induced contractile responses in pulmonary arterial systolic pressure from MCT-pulmonary hypertensive rats. | After day 42 in resveratrol therapy, there were no apparent elevations in circulating interleukins 2, 4, or 6, TNF-α, IFN-γ, MP-1α, MCP-1 or G-CSF caused by MCT. | Development of MCT-induced pulmonary hypertension is associated with transcriptional downregulation of atrong1, MuRF-1, eNOS and Kv1.5 mRNA expression. | n.e |
| Ba-Restier et al. [3] (2012) | Resveratrol led to significant attenuation in systolic and mean right ventricular pressures in monocrotaline injected rats; Resveratrol normalized the magnitude of right ventricular contractility (+dP/dtmax) to the level of controls; Resveratrol reversed both right ventricular hypertrophy and pronounced leftward septal deviation in monocrotaline treated rats. | Monocrotaline induced a progressive increase in 99mTc-Annexin binding in lung apoptosis cells compared to saline controls. Resveratrol treatment abrogated this increase in 99mTc-Annexin in monocrotaline-treated rats. | n.e | n.e |
| Chen et al. [36] (2014) | Hypobaric hypoxia exposure for 14 days increased the right ventricular hypertrophy measured by the RV/(LV+S) ratio. Treatment with 14 days of resveratrol normalized the chronic hypoxia-induced right ventricular hypertrophy in the neonatal rats. | Nitrite levels were measured from the media of normoxic and hypoxic hPASMC treated with resveratrol. Hypoxia significantly decreased nitrite levels relative to normoxia. However, resveratrol treatment no showed significant results on nitrite levels. | n.e | n.e | The addition of resveratrol for 48h prevented hypoxia-induced arginase II protein expression in all doses evaluated; Resveratrol did not affect arginase I protein levels in either normoxia or hypoxia; Treatment with resveratrol prevented the hypoxia-induced arginase II mRNA expression after 24h; Resveratrol did not affect arginase I mRNA expression in normoxia or hypoxia; hPASMC proliferation in vitro: hPASMC were evaluated for apoptosis by measuring cleaved caspase 3. There was no difference in cleaved caspase 3 protein expression in normoxia or hypoxia with or without resveratrol treatment; Inhibition of Akt phosphorylation prevents hypoxia-induced arginase II protein expression, result evidenced in all resveratrol doses evaluated. | n.e |

(Table 2) Contd…
| Author (year) | Cardiovascular Function | Endothelial Function | Inflammatory Markers | Oxidative Markers | Others Variables |
|---------------|-------------------------|---------------------|----------------------|------------------|------------------|
| Zhou et al. [41] (2015) | Resveratrol significantly prevented mPAP from increasing in both the 2.5 mg.kg⁻¹ day⁻¹ group and 20 mg/kg/day groups at both 14 and 21 days; Treatment with resveratrol decreased RVHI in a dose-dependent manner; Resveratrol attenuated the muscularization of intracinar arteries; 2.5 mg/kg/day and 20 mg/kg/day of resveratrol attenuated the increase of medial wall thickness. | n.e | n.e | n.e | PDGF-BB (10 ng/mL) treatment decreased expression of p21 but increased cyclin D1 and cyclin E expression in HPASMCs; Flow cytometry analysis showed significant G0/G1 accumulation of hPASMCs with SIRT1 overexpression 12 hours after PDGF-BB treatment; Resveratrol Increases SIRT1 and p21 Expression but Decreases Cyclin D1 Expression in Lungs of MCT-Induced PAH Rats; Resveratrol increased p21 expression but decreased cyclin D1 and cyclin E expressions after PDGF-BB stimulation. |
| Wilson et al. [26] (2016) | Resveratrol does not alter the weight heart, but the hypertrophy in the right ventricle was adjusted when compared with the control group. Resveratrol treatment was beneficial and reversed total heart surface area to a level observed in control hearts. This reduction in total heart surface area was attributed to a decrease in both total heart muscle area. | n.e | n.e | n.e | Resveratrol was beneficial and significantly reduced tunica media thickness in the pulmonary trunk compared with MCT-treated rats. |
| Xu et al. [18] (2016) | After 28 days of hypoxia exposure, the myocytes of the right ventricle in the hypoxia group were significantly enlarged versus the normoxic groups. The hypoxia exposure also markedly increased RVHI versus the normoxic groups and resveratol treatment significantly decreased the elevation of RVHI; The chronic hypoxic condition also dramatically increased RVSP versus the normoxic groups, and resveratrol treatment notably decreased the increased RVSP. Similarly, there was no significant difference found in the two normoxic groups. | n.e | Inflammatory factors IL-6, IL-1β, TNF-α, and cytokine VEGF were all significantly increased in mRNA levels after chronic hypoxia, and all these factors were decreased considerably after resveratrol treatment. | Compared to the normoxic groups, there was a significant elevation of H₂O₂ found in rat lungs after 28 days of hypoxic exposure, and resveratrol treatment significantly reduced its height in a dose-dependent way. | Chronic hypoxia exposure resulted in thickened pulmonary arterial tunica media and accumulated extracellular matrix and resveratrol treatment significantly reduced this process in a dose-dependent way. |
| Guan et al. [39] (2017) | Resveratrol inhibits the development of experimental PAH per hypoxia. | n.e | n.e | n.e | Migration of PASMCs in the resveratrol-treated group was reduced compared with the cells treated with hypoxia, and this effect was dose-dependent, inhibiting hypoxia; Protein expression levels of AKT were increased significantly in the hypoxic group compared with the 10 and 30 µmol/l resveratrol treated groups, and this effect was dose-dependent; Resveratrol inhibits hypoxia-induced proliferation and migration of PASMCs by inhibiting the PI3K/AKT signaling pathway; The protein level of p Akt was significantly suppressed by resveratrol. |

(Table 2) Contd…
### Effects of Resveratrol in Different Models of Pulmonary Arterial Hypertension

| Author(s) | Cardiovascular Function | Endothelial Function | Inflammatory Markers | Oxidative Markers | Others Variables |
|-----------|-------------------------|----------------------|----------------------|---------------------|------------------|
| Yu et al. [31] (2011) | Resveratrol improves right ventricular systolic pressure and mitigate RVHI. | n.e | n.e | n.e | Resveratrol reverses pulmonary vascular remodeling and contributes to the improvement of mitochondrial dysfunction. |
| Shi et al. [28] (2010) | Resveratrol inhibits the development of experimental PAH; The RVSP and right ventricular hypertrophy index decrease in MCT-treated rats after treatment with resveratrol; The combination resveratrol with two agents (resveratrol+PF543 or resveratrol+PDTC) in rats MCT-treated decreased significantly RVSP and RVHI. | n.e | n.e | n.e | The SphK1, cyclin D1, and S1P protein levels were decreased in resveratrol-treated PAH rats, as well as these same variables were decreased after the combination of resveratrol with two agents (resveratrol+PF543 or resveratrol+PDTC). |

Note: MCT: monocrotaline; PASMC: artery smooth muscle cells; PCNA: proliferating cell nuclear antigen; eNOS: endothelial nitric oxide synthase; IL: interleukin; TNF-α: Tumor necrosis factor α; PDGF-α: α-receptor for platelet-derived growth factor; PDGF-β: β-receptor for platelet-derived growth factor; TGF-β: transforming growth factor β; MCP-1: monocyte chemoattractant protein-1; NAD(P)H oxidase: phagocyte nicotinamide adenine dinucleotide phosphate oxidase; NOX-1: hydrogen peroxide; n.e: not evaluated; KCl: potassium chloride; IFN-γ: interferon-γ; MIP-1α: macrophage inflammatory protein-1α; G-CSF: granulocyte colony-stimulating factor; MuRF-1: muscle ring finger 1; K+ channel: messenger ribonucleic acid; hPASMC: human pulmonary artery smooth muscle cells; mRNA: messenger ribonucleic acid; mPAP: mean pulmonary artery pressure; RVHI: right ventricular hypertrophy index; PDGF-BB: platelet-derived growth factor-BB; SIRT1: silent information regulator 1; PAH: pulmonary arterial hypertension; RVSP: right ventricular systolic pressure; VEGF: vascular endothelial growth factor; H2O2: hydrogen peroxide; PASMC: pulmonary artery smooth muscle cells; P3K: phosphoinositide 3-kinase; Akt: Protein kinase B; RVHI: right ventricular hypertrophy index; PF543: drug that act like a specific inhibitor of sphingosine kinase 1; PDTC: pyrrolidine dithiocarbamate, an anti-oxidant; SphK1: sphingosine kinase 1.

### 4. DISCUSSION

Resveratrol (RES) is a phenolic compound naturally occurring in a variety of plants such as grapes and peanuts [34, 35]. Recent studies showed that RES presents a series of cardioprotective effects, including anti-inflammatory, antioxidant, anti-proliferative properties, as well as anti-atherosclerosis and anti-hypertensive action [5, 30, 36-38]. Furthermore, evidence suggests that RES could attenuate the developed of PAH once this substance acts on pathophysiological mechanisms of the disease such as vascular remodeling, inhibition of proliferation of the intima and media, inhibition of endothelial dysfunction, decreased vasoconstriction and activation of inflammatory processes [5, 30, 39-44]. Thus, this review article aimed to investigate the effects related to the dose of resveratrol in different models of pulmonary arterial hypertension.

The studies presented evidenced significant results on the anti-inflammatory response of RES in myocardial cells as observed at doses of 3 mg/kg, 25 mg/kg, and 40 mg/kg. It is recognized that inflammation is one of the main pathogenic mechanisms in the PAH as well as in other cardiovascular diseases [24, 45]. The inflammatory process in pulmonary arteries can injure pulmonary arterial endothelial cells, stimulating the proliferation of Pulmonary Arterial Smooth Muscle Cells (PASMC) [22]. However, it is observed that cytokines such as interleukin-1 and interleukin-6, are important mediators of inflammation that will contribute to the development of PAH [46, 47]. Still, we highlight that the dosage 3 mg/kg and 25 mg/kg were used in less than 21 days, both in experimental models induced by monocrotaline [38, 48].

The chemoprotective properties of RES can be observed on oxidative stress parameters that were significantly reduced also at doses of 3 mg/kg, 25 mg/kg, and 40 mg/kg, noting that these results were found in different models of PAH (monocrotaline and normoxia/hypoxia) and the dosage of 40 mg/kg not only reduced the plasma levels of the cytokines but also decreased the expression of their mRNA significantly. In a previous study, it was evidenced that the free radical-scavenging capacity of resveratrol depends on concentration. The author showed that the antioxidant properties of resveratrol are increased with higher levels of this nutraceutical [49].

Anti-proliferative effects of RES in PASMCs were observed at doses of 10 μmol/L, 30 μmol/L, 40 μmol/L, 50 μmol/L, 100 μmol/L, 2.5 mg/kg, 20 mg/kg, and 100 mg/kg. Studies have demonstrated that apoptosis of PASMCs is necessary for the regression of pulmonary vascular changes in the developed of PAH [50, 51]. However, the precise mechanisms related to the proliferation and apoptosis of these cells in the pathogenesis of PAH remain unclear. In this sense, Guan et al. (2017) [52] showed that resveratrol might inhibit the hypoxia-induced proliferation and migration of PASMCs, inhibiting the phosphatidylinositol 3-kinase (P13K) and protein kinase B (Akt) signaling pathway, thus attenuating pulmonary arterial remodeling. It is important to emphasize that the anti-proliferative effects of RES were observed in a relatively low dose (10-100 μmol/L) in a dose-dependent manner.

Regarding right ventricular hypertrophy was observed significant differences at doses of 40 μmol/L, 80 μmol/L, 100 μmol/L, 3 mg/kg, 25 mg/kg, and 100 mg/kg. We were emphasizing that the low doses (40-100 μmol/L) and high dose (100 mg/kg) were effective against hypertrophy in myocardial cells of neonatal rats exposed to hypoxia. The relationship between right ventricular hypertrophy and monocrotaline-induced PAH is well established [53]. However, Zhou et al. [54] showed the role of hypoxia in the development of right ventricular hypertrophy and the preven-
tive effect of RES on this parameter. Since, that acute or chronic alveolar hypoxia is a potent stimulus for pulmonary vasoconstriction leading to the proliferation and migration of PASMCs and thickening of the vessel wall, as well as compensatory right ventricular hypertrophy.

The benefits of the cardiovascular function were evidenced in all the studies included in this review. Thus, we observed that in lower doses (10-100 µmol/L) and high doses (2.5-100 mg/kg), RES protects in a dose-dependent manner against the development of PAH-induced through monocrotaline, normoxia, and hypoxia models. Therefore, the authors show improvements in variables such as right ventricular hypertrophy (RVH), right ventricular hypertrophy index (RVHI), right ventricular systolic pressure (RVSP), mean pulmonary artery pressure (mPAP), and right ventricular contractility (+dP/dt max). In this sense, we observe that such results also benefit the endothelial function, promoting reduction of pulmonary cells apoptosis, an increase of acetylcholine-induced relaxation, and improved endothelial nitric oxide synthase (eNOS) expression [30, 55]. These results demonstrate the efficacy of RES as a primary therapy in the treatment of PAH, promoting cardiovascular benefits through their chemoprotective, anti-inflammatory, antioxidant, and anti-proliferative properties, in different experimental models in a dose-dependent manner.

CONCLUSION

The results observed here showed that resveratrol, in low and high doses, protects PAH-induced through different models, as well as possesses chemoprotective, anti-inflammatory, antioxidant, and anti-proliferative properties. In the case of pulmonary injury related to PAH, resveratrol protects cells from apoptosis, thereby working as an anti-apoptotic agent. Therefore, we can observed that resveratrol presents protective heart action still in a relatively low dose (10-100 µmol/L). Besides, the results indicate that this polyphenol provides cardiovascular benefits, attenuating the development of PAH in a dose-dependent manner.

LIST OF ABBREVIATIONS

PAH = Pulmonary Arterial Hypertension
RES = Resveratrol
PASMC = Pulmonary Arterial Smooth Muscle Cell
MCT = Monocrotaline
HPH = Hypoxia-induced Pulmonary Hypertension
hPASMC = Human Pulmonary Artery Smooth Muscle Cells
mPAP = Mean Pulmonary Artery Pressure
RVHI = Right Ventricular Hypertrophy Index
RVHI = Right Ventricular Hypertrophy Index
RVSP = Right Ventricular Systolic Pressure

AUTHORS’ CONTRIBUTIONS

ACF, JSS, RD, JMPC, AWSM and ASMV: conducted literature research and wrote the draft of the manuscript.

CAADF, CID and MRQB provided editorial input and revision for the final version. CTM and JOB: read and approved the final manuscript.

CONSENT FOR PUBLICATION

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The authors declare no conflict of interest, financial or otherwise.

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Effects of Resveratrol in Different Models of Pulmonary Arterial Hypertension

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