

# Inhibition of poly (ADP-ribose) Polymerase-1 (PARP-1) improves endothelial function in pulmonary hypertension

Mohammad Shafiq<sup>a,b,c,1</sup>, Zahid Rasool Lone<sup>a,1</sup>, Adam Olaitan Abdulkareem<sup>a,b,d</sup>,  
Gurpreet Kaur<sup>a</sup>, Sai Navya<sup>a</sup>, Himalaya Singh<sup>a,b</sup>, Kumaravelu Jagavelu<sup>a,b</sup>, Kashif Hanif<sup>a,b,\*</sup>

<sup>a</sup> Division of Pharmacology, CSIR-Central Drug Research Institute, Lucknow, 226031, India

<sup>b</sup> Academy of Scientific and Innovative Research, Ghaziabad, Uttar Pradesh, India

<sup>c</sup> Department of Pediatrics, Ann & Robert H. Lurie Children's Hospital, Chicago, IL, USA

<sup>d</sup> Animal Physiology Unit, Department of Zoology, University of Ilorin, Nigeria

## ARTICLE INFO

### Keywords:

PARP-1

Endothelial dysfunction

Pulmonary hypertension

DNA damage

1, 5-Isoquinolinediol

## ABSTRACT

Endothelial dysfunction is critical in the pulmonary vasculature during pulmonary hypertension (PH). Moreover, in PH, increased inflammation and oxidative/nitrosative stress cause DNA damage, activating poly (ADP-ribose) polymerase-1 (PARP-1). Meloche et al. (2014) and our previous research have shown that inhibiting PARP-1 is protective in PH and associated RV hypertrophy. However, the role of PARP-1 in pulmonary arterial endothelial dysfunction has not been explored completely. Therefore, the current study aims to investigate the involvement of PARP-1 in endothelial dysfunction associated with PH. Hypoxia (1% O<sub>2</sub>) was used to induce a PH-like phenotype in human pulmonary artery endothelial cells (HPAECs), and PARP-1 inhibition was achieved via siRNA (60 nM). For the in vivo study, male Sprague Dawley rats were administered monocrotaline (MCT; 60 mg/kg, SC, once) to induce PH, and 1, 5-isoquinolinediol (ISO; 3 mg/kg) was administered daily intraperitoneally to inhibit PARP-1. PARP-1 inhibition decreased proliferation and inflammation, as well as improved mitochondrial dysfunction in hypoxic HPAECs. Furthermore, PARP-1 inhibition also promoted apoptosis by increasing DNA damage in hypoxic HPAECs. In addition, inhibition of PARP-1 reduced cell migration, VEGF expression, and tubule formation in hypoxic HPAECs. In in vivo studies, PARP-1 inhibition by ISO significantly decreased the RVP and RVH as well as improved endothelial function by increasing the pulmonary vascular reactivity and expression of p-eNOS in MCT-treated rats.

## 1. Introduction

Pulmonary endothelial dysfunction plays a central role in the development and progression of pulmonary hypertension (PH), a severe cardio-pulmonary condition characterized by chronic vasoconstriction, endothelial dysfunction, and structural remodeling of pulmonary vessels [1]. Current PH treatments mostly provide symptomatic relief due to their vasodilatory nature and lack of antiproliferative and proapoptotic properties [2]. Therefore, research focusing on preventing or reversing pulmonary vascular remodeling and complicated lesion development by regulating underlying factors is essential. Pulmonary vascular remodeling during PH is significantly determined by endothelial dysfunction

apart from pulmonary artery smooth muscle cell disruption (PASMCs), as reported in our previous work [3].

PH is linked to severe inflammation and oxidative/nitrosative stress results in a toxic environment for cells, as well as the potential for DNA damage [4]. Endothelial dysfunction begins with endothelial damage caused by hypoxia and toxins, which initially increase apoptosis but later promote the development of apoptosis-resistant and endothelial cell hyperproliferation [3,5]. Endothelium in normal conditions is in a quiescent and stable state but various factors like endothelial injury, shear stress, hypoxia, inflammation, and genetic factors cause it to become hyperactive. This, in turn, triggers endothelium to secrete various growth factors like vascular endothelial growth factor (VEGF)

**Abbreviations:** PARP-1, poly (ADP-ribose) polymerase-1; ED, Endothelial Dysfunction; ISO, 1, 5-isoquinolinediol; PH, Pulmonary Hypertension.

\* Corresponding author. Division of Pharmacology, CSIR-Central Drug Research Institute, Sector-10, Jankipuram Extension, Sitapur Road, Lucknow, 226031, Uttar Pradesh, India.

E-mail address: [k\\_hanif@cdri.res.in](mailto:k_hanif@cdri.res.in) (K. Hanif).

<sup>1</sup> Authors contributed equally.

<https://doi.org/10.1016/j.pupt.2023.102200>

Received 22 August 2022; Received in revised form 2 February 2023; Accepted 15 February 2023

Available online 25 February 2023

1094-5539/© 2023 Elsevier Ltd. All rights reserved.

and fibroblast growth factor 2 (FGF 2), vasoconstrictive factors like thromboxane and endothelin-1 (ET-1), and reduce the secretion of vasodilators, like nitric oxide (NO) and prostacyclin; all these contribute to the endothelial dysfunction and loss of homeostatic functions [6]. Moreover, excessive disrupted endothelial cell proliferation, apoptosis resistance, and concurrent neo-angiogenesis culminate in the formation of plexiform lesions, which are typical clinical characteristics of the pulmonary arteries in individuals with pulmonary arterial hypertension [7,8].

Emerging research indicates that DNA damage signaling underlies pulmonary vasculature changes in PH [4]. Studies have reported that inflammation as well as oxidative/nitrosative stress such as that occurs in pulmonary hypertension induce endothelial cell DNA damage by causing single and double-strand breaks and activating poly (ADP-ribose) polymerase-1 (PARP-1) [9,10]. PARP-1 is a nuclear DNA-repairing enzyme that participates actively in DNA repair by generating substantial amounts of poly (ADP-ribose) along the break site using nicotinamide adenine dinucleotide (NAD) [11]. However, hyper-activation of this enzyme by inflammation or oxidative/nitrosative stress causes severe depletion of NAD and subsequent adenosine triphosphate (ATP), resulting in cellular energy depletion, permanent cytotoxicity, and even cell death [12]. PARP-1 activity inhibition has been shown to limit pathologies associated with smooth muscle and endothelial dysfunction [9]. Moreover, PARP-1 is involved in many other aspects of PH like NF- $\kappa$ B mediated transcription of various genes including interleukine-6 (IL-6), IL-1 $\beta$  and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) [13]. In addition, IL-6 upregulation caused by the activation of PARP-1 activates signal transducer and activator of transcription 3 (STAT3), which, in turn, can contribute to the nuclear factor of activated T cells (NFAT) and hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) activation, promoting the apoptotic resistance that defines endothelial dysfunction in PH [10]. Pharmacological inhibition of PARP-1 in animal models of hypertension, dyslipidemia, aging, and diabetes has been shown to improve endothelial dysfunction and is currently considered a new therapeutic approach [14].

An earlier study from our lab demonstrated that inhibiting PARP-1 significantly reverses increases in right ventricular pressure and right ventricular hypertrophy in both preventive and curative treatment in monocrotaline (MCT)-injected rats by lowering oxidative and nitrosative stress, inflammation, mitochondrial dysfunction, and restoring the balance of matrix metalloproteinases/tissue inhibitors of metalloproteinases (MMPs/TIMP2) expression [15]. These findings were consistent with the findings of Meloche et al., who found that treatment with the PARP-1 inhibitor ABT-888 reversed right ventricle pressure (RVP) and right ventricle hypertrophy (RVH) in Sugan-hypoxia and MCT rat models of PH. Furthermore, they also reported that PARP-1 inhibition improved pulmonary vascular remodeling associated with PH by reducing cytokine production as well as increasing miR-204 and, as a result, decreasing NFAT and HIF-1 $\alpha$  activation in Pulmonary artery hypertension-pulmonary artery smooth muscle cells (PAH-PASMCs) [10]. In addition to these, it has also been demonstrated that PARP-1 modulates angiogenesis, since inhibiting PARP-1 reduced endothelial cell proliferation and neo-angiogenesis, implying an anti-angiogenic role in cancer [16].

However, the involvement of PARP-1 in pulmonary artery endothelial cell proliferation, apoptosis-resistance as well as neo-angiogenesis, all of which are critical factors in endothelial dysfunction, has not been investigated in PH. Using human pulmonary artery endothelial cells (HPAECs) and distal pulmonary arteries; we demonstrate for the first time that PARP-1 overexpression is associated with endothelial dysfunction in PH. In hypoxic HPAECs, siRNA-mediated PARP-1 inhibition reduced cellular proliferation, apoptosis resistance, and mitochondrial dysfunction as well as prevented the formation of neo-angiogenesis by inhibiting endothelial cell migration and tubule formation. Furthermore, in the MCT rat model of PH, pharmacological inhibition of PARP-1 by 1,5-Isoquinolinediol (ISO) preserved

endothelial function by regulating eNOS.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Antibiotic/antimycotic solution (15240062), trypsin (25200056), and DAPI (D1306) were purchased from Invitrogen (Carlsbad, CA, USA). 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl benzimidazole carbocyanine Iodide (JC-1; 420,200). Primary antibodies to NF- $\kappa$ B (ab16502), Histone H3 antibody (ab201456), eNOS (ab300071), p-eNOS (ab76199), and COX4 (ab110272) were from Abcam (USA),  $\beta$ -actin (A5441) from Sigma (USA), PCNA (sc-25280), Bcl2 (sc-7382), Bax (sc-7480), PARP-1 (sc-1561), AIF (sc-13116) Cytochrome c, (sc-13156) and VEGF (sc-7269), Cyclin D1 (sc-8396), p53 (sc-393031) from Santa Cruz (USA), HIF-1 $\alpha$  (P1-16601) from Thermo Fisher Scientific (USA),  $\gamma$ H2AX (AF6187), from Affinity Biosciences, Alexa Fluor 594 and Alexa Fluor 488 IgG conjugate (Invitrogen) and secondary HRP-conjugated antibodies were purchased from Santa Cruz Biotechnology (USA). ELISA kits for TNF- $\alpha$  (DY210-05), IL-6 (DY206-05), and IL-10 (DY217B-05) were procured from the R&D system, Minneapolis, Minnesota, USA.

### 2.2. Cell culture

Human pulmonary artery endothelial cells (HPAECs; catalog no. CC-2530) were purchased from Lonza and cultured in Endothelial Cell Growth Medium-2 BulletKit (EGM-2, catalog no. CC3162, Lonza, USA). In this study, HPAECs were used in passages 3–8.

### 2.3. Hypoxia exposure

The HPAECs were subjected to a hypoxic environment for 72 h in a chamber with (1% O<sub>2</sub>, 5% CO<sub>2</sub> and balance N<sub>2</sub>) to induce proliferation, which was validated by measuring the expression of hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ). For PARP-1 inhibition, cells were treated for 24 h with 60 nM PARP-1 siRNA and Scrambled siRNA (catalog No. sc-29437 and sc-37007 respectively), following the manufacturer's protocol. After 24 h of treatment, the media was changed, and the hypoxia exposure was continued for up to 72 h [3].

### 2.4. Assessment of cell proliferation

The proliferation of HPAECs was determined by performing a cell counting assay. In brief, HPAECs were seeded at a density of 5000 cells/well in a 6-well plate and incubated at 37 °C (5% CO<sub>2</sub>, 95% N<sub>2</sub>) with 100% relative humidity. After 24 h, cells were exposed to hypoxia or normoxia (with or without PARP-1 siRNA treatment) for 72 h. Cells were harvested with trypsin and counted with a hemocytometer under a bright-field inverted microscope at the end of the experiments (DMI8, Leica Wetzlar, Germany) [17].

### 2.5. PARP-1 activity

PARP-1 activity was estimated by using a commercially available Universal colorimetric PARP-1 assay kit (Trevigen, Gaithersburg, Maryland, USA), according to the manufacturer's protocol [15].

### 2.6. Caspase 3 activity

The extent of apoptosis in HPAECs was determined by using a commercially available caspase-3 assay kit (Biovision, Milpitas, CA, USA), according to the manufacturer's instructions [3,15].

### 2.7. Endothelial cell migration assay

The scratch assay was used to assess the migration ability of HPAECs,

as previously described [3]. In brief, cells were seeded in a 6-well plate and grown to 80–90% confluence. The cells were serum-starved overnight before being wounded by scratching lengthwise using a sterile tip (200  $\mu$ l). The cells were photographed at time 0 h and then subjected to normoxia or hypoxia (1% O<sub>2</sub>) for 72 h. After 72 h, cell migration was determined by photographing the cells again. The ImageJ software was used to measure the change in distance between the cell fronts as compared with the time 0 h photograph. The data is presented as a fold change from normoxia [3].

## 2.8. Tubule formation

The matrigel tube formation assay was used to determine the angiogenic property of HPAECs, as previously described [3]. In brief, cells were seeded in a 96-well plate and subjected to hypoxia for 72 h, either with or without PARP-1 siRNA treatment. The cells were then trypsinized and added  $1 \times 10^3$  cells/well in a 96-well plate pre-coated with matrigel and incubated under normoxia or hypoxia for 6 h. The images were captured using a fluorescence microscope (Leica DM 6000, Wetzlar, Germany), and the total tube length was counted and analyzed using Leica Qwin software [3].

## 2.9. Comet assay

Comet assay was performed in dark to analyze DNA damage, according to the previously described method, with some modifications [18]. In brief, low melting point (LMP, 0.8%) agarose was prepared in saline and kept at 39 °C to avoid solidification. Cells were subjected to hypoxia and treated with either scrambled siRNA or PARP-1 siRNA. At the end of the treatment, cells were trypsinized, rinsed with PBS, and then gently mixed with 250  $\mu$ l of 0.8% LMP agarose. The resulting cell suspension was then layered onto the frosted surface of the slides and placed on ice for 5 min for agarose solidification. Following that, the slides were immersed in the pre-chilled lysis buffer for 1 h before being immersed in an alkaline buffer (pH, 13) for 20 min. Subsequently, electrophoresis was conducted at the rate of 0.6 V/cm for 30 min utilizing a compact power supply. To remove the alkali and detergents, the slides were gently washed with 0.4 M Tris (pH 7.5). The cells were stained with PI (20  $\mu$ g/ml) and the images were captured using a fluorescence microscope. About 60–80 cells were evaluated in each sample and the % tail DNA parameter was analyzed by CASP software.

## 2.10. Estimation of cytokine level

Following hypoxia exposure, the levels of cytokines (TNF-, IL-6, and IL-10) in the culture supernatant of HPAECs were determined by ELISA using specific kits (R & D system, Minneapolis, Minnesota, USA), according to the manufacturer's protocol [17,19].

## 2.11. Isolation of mitochondrial/cytoplasmic fraction

Mitochondrial proteins were isolated from the cytosolic fraction using a commercially available kit from Abcam (catalog number-ab65321, USA), as specified by the manufacturer.

## 2.12. Isolation of nuclear/cytoplasmic fraction

The nuclear and cytoplasmic fractions were separated according to the manufacturer's protocol using a commercially available kit from Biovision (catalog number- K266-25, USA).

## 2.13. Assessment of mitochondrial membrane potential ( $\Delta\psi_m$ )

The difference in mitochondrial membrane potential ( $\Delta\psi_m$ ) was measured by flow cytometry using the JC-1 dye, as previously described [3]. During physiological conditions, the dye accumulates in the

mitochondrial matrix due to the mitochondrial membrane potential, forming J-aggregates with characteristic absorption and emission spectra that can be measured by FACS. At the end of the experiment, HPAECs were incubated with JC-1 dye (2.5  $\mu$ M) in a culture medium at 37 °C for 30 min. The cells were harvested using trypsin and washed twice with PBS before being assessed for mitochondrial membrane potential using the FACS Calibur (FACS Calibur, BD Bioscience).

## 2.14. Estimation of mitochondrial reactive oxygen species (mROS)

Mitoxox was used to determine mROS in hypoxic HPAECs, as previously described [19]. The HPAECs were treated with scrambled siRNA or PARP-1 siRNA after being exposed to hypoxia. At the end of the treatment, cells were incubated with 2.5  $\mu$ M Mitoxox for 30 min at 37 °C in a culture medium. Trypsin was used to harvest the cells, which were then washed twice with PBS. Mitochondrial ROS was assessed using FACS Calibur (FACS Calibur, BD Bioscience).

## 2.15. Estimation of cellular ATP and NAD

The ENLITEN ATP Assay System Bioluminescence Detection Kit and NAD/NADH Cell-Based Assay Kit (Cayman Chemical, Ann Arbor, MI, USA) were used to estimating cellular ATP and NAD levels, following the manufacturer's protocol [17].

## 2.16. RNA preparation and RT-PCR

As described previously, quantitative gene expression analysis was performed using SYBR Green technology [17]. In brief, for cDNA synthesis, 1  $\mu$ g of total RNA was processed as per the manufacturer's protocol (cDNA synthesis kit catalog no. 4368814, Thermo Fisher Scientific). Using cDNA as a template, several genes like BRD2 (Forward-TGCTCGTGAGGGGAATACA and Reverse- TTTGGGATTGGACACCTCCG), BRD4 (Forward-GTTGATGTGATTGCCGGCTC and Reverse-TGCACGTGACTGTGATACGG) and Chk1 (Forward-CGTTCCGCCGAAAGCATTTC and Reverse-GAGCACCTCGGCATTTC) (Integrated DNA Technologies, IDT) were amplified using Light Cycler 480 (Roche, USA) and SYBR green chemistry (Thermo Scientific, USA). Expression of each target gene was normalized to  $\beta$ -actin and the data were expressed as fold changes.

## 2.17. Animal studies

Male Sprague-Dawley (SD) rats 8–10 weeks of age, weighing 270–300 g, were procured from the National Laboratory Animal Center of the CSIR-Central Drug Research Institute, Lucknow, India. Rats were handled in accordance with the guidelines approved by the Institutional Animal Ethics Committee (IAEC), CSIR-Central Drug Research Institute, Lucknow (IAEC/2017/281). Rats were divided into four groups (control, MCT, MCT + Dimethylsulfoxide (DMSO) and MCT+1, 5-Isoquinolinediol (ISO), 6 rats in each group). As previously described [17], MCT (60 mg/kg, s.c., once) was used to induce pulmonary hypertension in rats. PARP-1 inhibition was achieved with daily i.p. administration of ISO (3 mg/kg) for 35 days. The rats were euthanized by urethane (1.5 g/kg) at the end of the experiment.

## 2.18. Measurement of systemic hemodynamic parameter and right ventricle pressure (RVP)

RVP was determined 35 days after MCT treatment. In brief, the rats were anesthetized with urethane (1.5 g/kg; i.p.), placed on an isothermal pad, and artificially ventilated during surgical operations to preserve normal body temperature and physiology. The RVP was measured by opening the abdomen and introducing a fluid-filled catheter into the RV, which was then connected to a pressure transducer coupled to a data acquisition system via a 25-gauge blunted needle. All

animals were allowed to stabilize before final readings were taken. After measuring right ventricular blood pressure, the rats were sacrificed through transcardiac perfusion [15,17].

### 2.19. Measurement of organ weight

Following the measurement of hemodynamic parameters, rats were sacrificed and the RV wall, left ventricular (LV) wall and ventricular septum (S) were separated from the hearts. The Fulton index (RV/LV + S) and the RV/heart weight (HW) ratio were determined as markers of hypertrophy.

### 2.20. Study of pulmonary endothelial function

The lungs of the rats were quickly removed and the two segments of the extra lobar pulmonary artery were dissected. The 4 mm wide (transverse) pulmonary artery rings were hung between two hooks in organ baths containing Kreb's solution for 90 min at 37 °C and oxygenated continuously. The pulmonary arterial rings were held under constant tension of 1 g throughout the experiment. The response was measured before and after the experimental protocol in each pulmonary ring. Force transducers (FSG-01; Experimetria, Budapest, Hungary) and a S.P.E.L. solution pack from Experimental Laboratories ADVANCE ISOSYS data acquisition and analysis software (Charles-River Laboratories, Hungary/Germany, Experimetria Ltd., Budapest, Hungary) were used to obtain isometric measurements. To assess the maximum tissue contractility and viability, the pulmonary artery rings were subjected to an 80 mM KCl solution. After that, 5-hydroxytryptamine (5-HT; 3 nM–3 mM) was used to constrict the pulmonary artery rings to generate a concentration-response curve. The functioning of the pulmonary artery endothelium was studied by measuring the relaxation response to acetylcholine (3 nM–3 mM) in 5-HT pre-constricted pulmonary artery rings. Acetylcholine relaxant responses were then plotted as a percentage of relaxation [19].

### 2.21. Western blot studies

Cells after the treatment were lysed and sonicated in cell lysis buffer for subcellular fractions (whole cell lysate and mitochondrial fraction) as reported previously [17]. Protein (40–60 µg) was separated on a 10% polyacrylamide gel with an equal amount of protein loaded for each of the experiments and transferred to the PVDF membrane. Subsequently, the PVDF membrane was blocked using 5% BSA in TBST (10 mM Tris, pH 7.5, 100 mM NaCl, and 0.1% Tween-20) for 2 h and then incubated with respective primary antibodies- PARP-1 (1:1000), YH2AX (1:1000), PCNA (1:1000), VEGF (1:2000), Bcl2 (1:1000), BAX (1:1000), NF-κB (1:2000), β-actin (1:2000), p-eNOS (1:2000), eNOS (1:2000), at 4 °C overnight. Following washing, the membrane was incubated with HRP-conjugated secondary antibodies (1:4000). The immunoreactive bands were detected using enhanced chemiluminescence (ECL) (Millipore, USA). My image analysis software (Thermo Scientific, USA) was used to determine band intensity using spot densitometry analysis.

### 2.22. Statistical analysis

For statistical analysis, Prism software version 8.0 (Graph Pad Software, San Diego, CA, USA) was used. The data were presented as mean ± SEM. One-way analysis of variance (ANOVA) was used to determine statistical significance, followed by Tukey's multiple comparison test. A value of  $p < 0.05$  was considered to be statistically significant.

## 3. Results

### 3.1. Hypoxia induces PARP-1 expression and cellular proliferation in hypoxic HPAECs

Hypoxia-Inducible Factor-1(HIF-1α) is regarded as the main transcriptional regulator of cellular and developmental response to hypoxia [20]. We previously found that 72 h of hypoxia was optimal for inducing HIF-1α expression in human pulmonary artery endothelial cells (HPAECs), a characteristic of the hypoxic environment inside cells [3]. Herein, we observed the same and found an increased expression of HIF-1α (Fig. 1A) in hypoxic HPAECs which was significantly reduced by the treatment with PARP-1 siRNA. Moreover, hypoxia also elevated the expression (Fig. 1A) and activity (Fig. S1) of PARP-1 in HPAECs which were brought down by silencing PARP-1 using siRNA.

In addition, scores of studies have demonstrated that hypoxia promotes cellular proliferation and causes a functional imbalance in the pulmonary endothelium [3,21,22]. Previously, PARP-1 inhibition has been shown to decrease proliferation in PAH-PASMCs [10]. Therefore, we studied the implication of PARP-1 on cellular proliferation in hypoxic HPAECs and found increased cell count (Fig. 1B) and PCNA (Fig. 1C) expression in hypoxic HPAECs which were significantly diminished by PARP-1 inhibition. In addition, expressions of cell cycle regulatory proteins were also studied and found that hypoxia increased the expression of Cyclin D1 and decreased that of p53 (Fig. 1D). However, PARP-1 inhibition significantly attenuated the expressions of both regulatory proteins. Furthermore, we also found the increased mRNA level of checkpoint kinase1 (Chk1) (Fig. 1E), a cellular proliferation marker, in hypoxic HPAECs which was significantly decreased by the treatment with PARP-1 siRNA. Altogether, these results revealed that PARP-1 is involved in the proliferation of HPAECs.

### 3.2. siRNA mediated PARP-1 inhibition decreases inflammation in hypoxic HPAECs

Inflammation plays a critical role in the onset and development of endothelial dysfunction in PH [6]. The hypoxia-exposed HPAECs showed elevated mRNA levels of BRD2 (bromodomain-containing protein 2) and BRD4 (Fig. 2A), as well as the expression of NF-κB (Fig. 2B) which were significantly reduced by PARP-1 inhibition. Furthermore, we also examined the levels of inflammatory cytokines in hypoxic HPAECs and found increased levels of pro-inflammatory cytokines, TNF-α (Fig. 2C), and IL-6 (Fig. 2D) as well as decreased levels of the anti-inflammatory cytokine, IL-10 (Fig. 2E). PARP-1 inhibition by siRNA significantly reduced pro-inflammatory cytokine levels while increasing the level of anti-inflammatory cytokine. All these findings indicate that PARP-1 modulates inflammation in hypoxic HPAECs.

### 3.3. PARP-1 inhibition modulates DNA damage and promotes apoptosis in hypoxic HPAECs

Oxidative stress and inflammation are the potent triggers of DNA damage during pulmonary hypertension which favors the pro-proliferative and apoptosis resistance phenotype in pulmonary vascular cells [4]. In the present study, a comet assay was performed to check the effect of siRNA-mediated inhibition of PARP-1 on DNA damage. There was a significant increase in DNA damage as evidenced by the increased percentage of tail DNA in hypoxic HPAECs as compared to normoxia. PARP-1 siRNA treatment further significantly aggravated the DNA damage (Fig. 3A), thereby pushing the hypoxic HPAECs toward apoptosis. Moreover, we also observed the increased expression of the DNA damage marker, γ-H2AX, in hypoxic HPAECs which was further increased by PARP-1 inhibition (Fig. 3B).

In addition, we investigated the fate of apoptosis in hypoxic HPAECs and found a significant apoptosis-resistance phenotype in comparison to normoxic cells. Hypoxia-induced HPAECs led to a reduced expression of

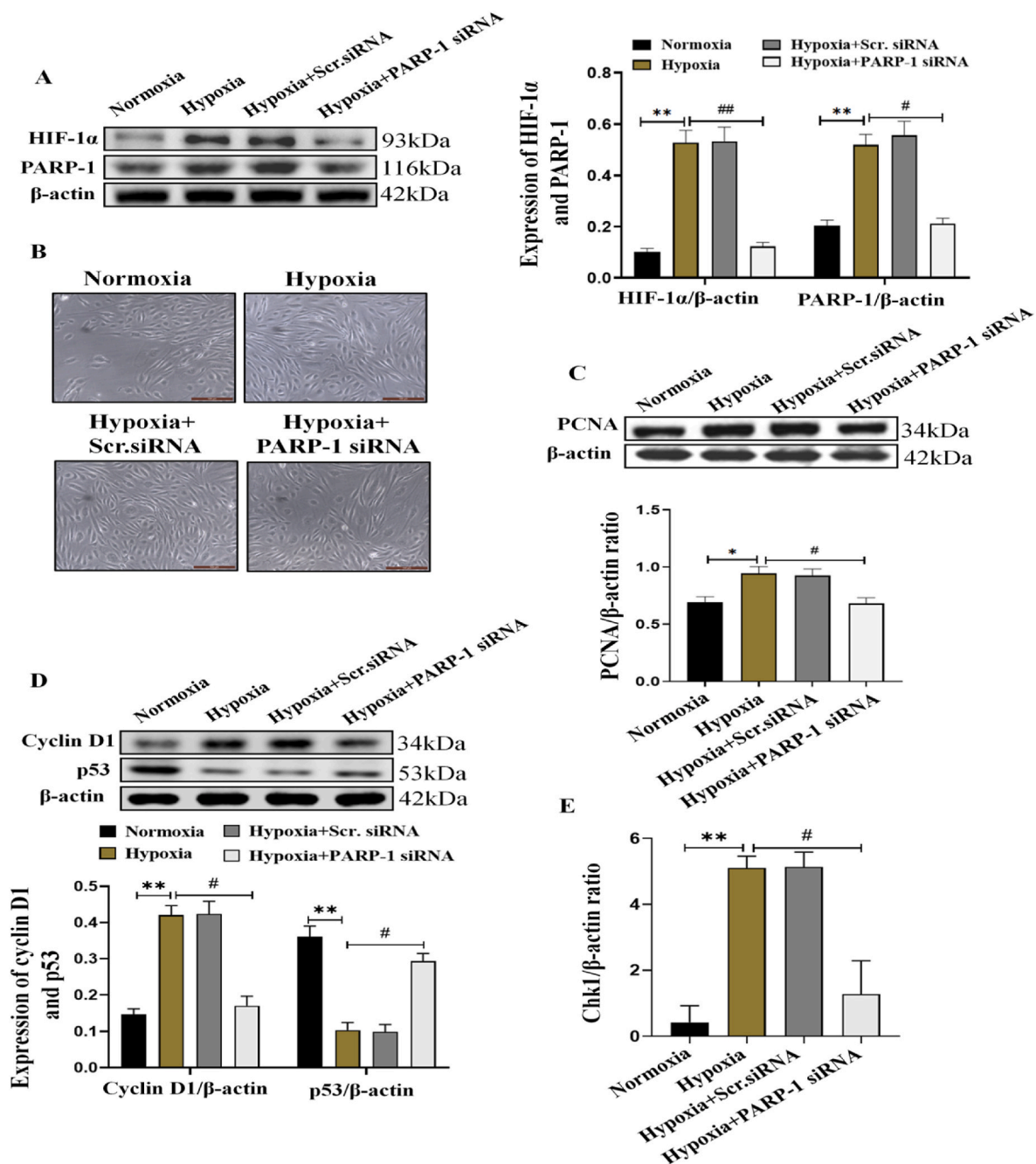


**Fig. 1.** PARP-1 inhibition reduced proliferation in hypoxic HPAECs. (A) Expression of HIF-1 $\alpha$  and PARP-1, (B) Cell count (Scale bar 100  $\mu$ m), (C) expression of PCNA, (D) expression of Cyclin D1 and p53, and (E) mRNA expression of Chk1 in hypoxic HPAECs. Data are shown as Mean  $\pm$  SEM (n = 3), \*Normoxia vs. Hypoxia, #Hypoxia vs. PARP-1 siRNA. \*\*P < 0.01, and #p < 0.05.

the pro-apoptotic protein, Bax, and increased expression of the anti-apoptotic protein, Bcl2 (Fig. 3C). Moreover, in hypoxic HPAECs, there was also a reduction in caspase 3 activity (Fig. 3D). PARP-1 inhibition with siRNA weakened the apoptosis resistance phenotype by promoting apoptosis as can be seen from the increased expression and activity of Bax and caspase-3, respectively while at the same time reducing the expression of Bcl2.

#### 3.4. PARP-1 inhibition reduces cell migration and tubule formation in hypoxic HPAECs

Hypoxia promotes angiogenesis by regulating VEGF expression which is a direct transcriptional target of HIF-1 $\alpha$  [23]. Also, PARP-1

inhibition or deletion has been shown to suppress angiogenesis in different types of cancers [16]. Therefore, to understand the involvement of PARP-1 in angiogenesis we performed a wound-healing assay (Fig. 4A) to evaluate the hypoxia-induced cell migration of HPAECs. There was increased endothelial cell migration in hypoxic HPAECs which was significantly reduced by PARP-1 siRNA treatment. Furthermore, endothelial cell migration is a critical step towards neo-vascularization; we, therefore, measured the percentage tubule formation (Fig. 4B) for the *in vitro* determination of neo-angiogenesis and found it to be significantly increased as compared to normoxic cells. Moreover, we also observed the increased expression of VEGF, another marker for angiogenesis, in hypoxic HPAECs as compared to normoxia ((Fig. 4C). However, silencing of PARP-1 by siRNA

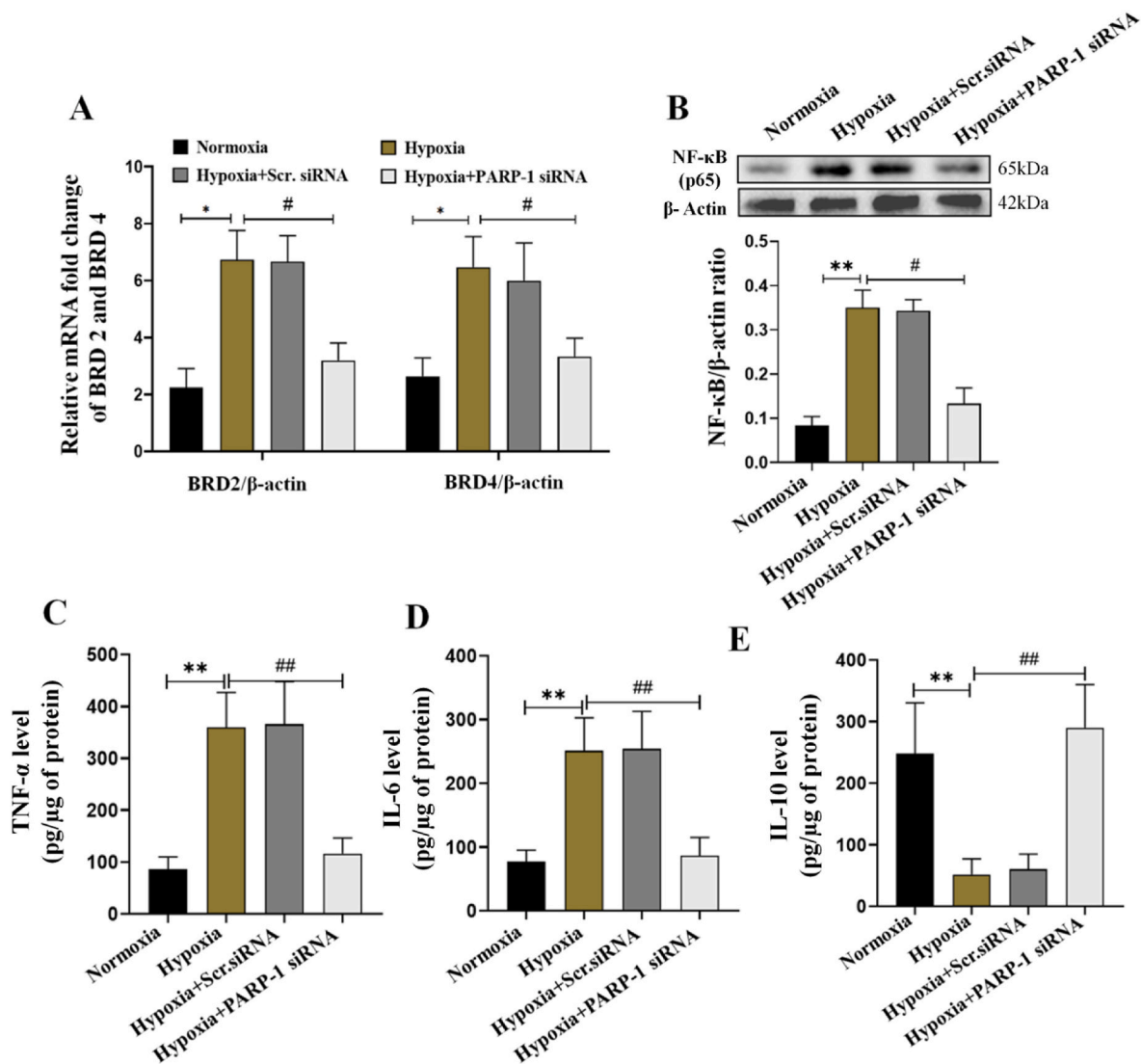


**Fig. 2.** Inhibition of PARP-1 by siRNA decreased inflammation in hypoxic HPAECs. (A) Relative fold change of BRD2 and BRD4 mRNA expression (B) expression of NF-κB (p65 subunit), (C) TNF-α level (ELISA) (D) IL-6 level (ELISA), and (E) IL-10 level (ELISA). Data are shown as Mean ± SEM (n = 3), \*Normoxia vs. Hypoxia, #Hypoxia vs. PARP-1 siRNA. \*\*P < 0.01, \*p < 0.05, ##p < 0.01, #p < 0.05.

significantly decreased the percentage of tubule formation as well as VEGF expression, thereby suggesting the improvement of endothelial functions by preventing angiogenesis.

### 3.5. Inhibition of PARP-1 improves mitochondrial function and apoptosis in hypoxic HPAECs

Hypoxia is known to cause mitochondrial dysfunction in PAECs by promoting hyperpolarization of the mitochondrial membrane and increasing mitochondrial reactive oxygen species (mROS) [3]. In corroboration with this, we also observed the increased levels of mROS (Fig. 5A) and mitochondrial membrane potential (Fig. 5B) in HPAECs exposed to hypoxia, as evidenced by increased MitoSOX positive cells and decreased JC-1 monomers, respectively. PARP-1 inhibition by siRNA improved the anomalies, thus indicating better preservation of mitochondrial functions.

Furthermore, mitochondrial membrane hyperpolarization suppresses apoptosis by blocking the voltage-dependent transition pores that release pro-apoptotic proteins [19]. We observed the same in our study that hypoxia inhibited the release of cytochrome c (cyt c) from mitochondria to cytosol, as evident from the increased expression of cyt

c in mitochondrial fraction and decreased expression in the cytosolic fraction of hypoxic HPAECs, thus promoting apoptosis resistance. Moreover, we also found increased expression of apoptosis-inducing factor (AIF) in mitochondrial fraction and decreased expression in the cytosolic fraction of hypoxic HPAECs which is needed for caspase-independent apoptosis. Inhibition of PARP-1 by siRNA significantly ameliorated the above effects and increased the translocation of cyt c and AIF from mitochondria to cytosol and nucleus respectively (Fig. 5C).

Furthermore, mitochondrial dysfunction and increased PARP-1 activity are also associated with the exhaustion of ATP and NAD levels, indicating a reduction in energy production [19]. Herein, we found reduced levels of ATP (Fig. 5D) and NAD (Fig. 5E) in HPAECs exposed to hypoxia, which were significantly increased by PARP-1 inhibition. Altogether, these studies suggest that PARP-1 inhibition preserved mitochondrial functions and improved energy production in hypoxic HPAECs.

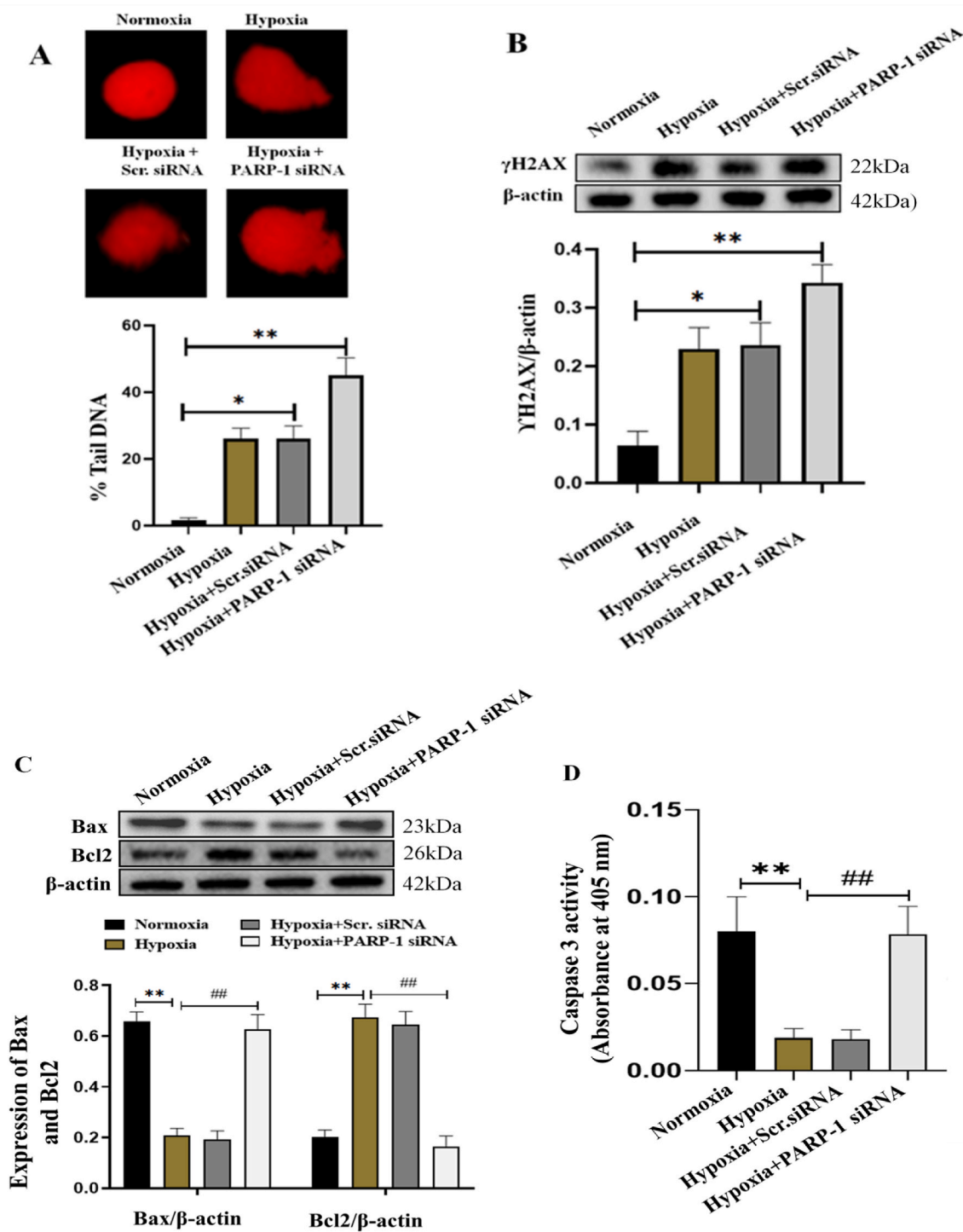


**Fig. 3.** siRNA mediated PARP-1 inhibition promoted apoptosis in hypoxic HPAEC. (A) Representative images and analysis of DNA damage using comet assay (B) expression of  $\gamma$ -H2AX, (C) expression of Bax and Bcl2, and (D) caspase 3 activity. Data are shown as Mean  $\pm$  SEM (n = 3), \*Normoxia vs. Hypoxia, #Hypoxia vs. PARP-1 siRNA. \*\*P < 0.01, \*p < 0.05, ##p < 0.01.

### 3.6. PARP-1 inhibition decreases right ventricular pressure (RVP) and RV hypertrophy (RVH) in MCT-treated rats

To determine the role of PARP-1 on RVP and RVH, we studied the expression of PARP-1 by immunostaining in the lung vasculature of MCT-treated rats. Results revealed a significantly increased expression of PARP-1 in the endothelial cells of pulmonary vessels as compared to control which was markedly reduced by the treatment with PARP-1 inhibitor, 1,5-Isoquinolinediol (ISO) (Fig. 6B). Furthermore, we also

observed the increased expression (Fig. 6C) and activity (Fig. 6D) of PARP-1 in distal pulmonary arteries of MCT-treated rats which were also reduced by the inhibition of PARP-1. Furthermore, induction of PH and RVH were evident in rats treated with MCT, as indicated by increased RVP and Fulton index (Figs. S2A–B). PARP-1 inhibition by ISO showed a robust decrease in RVP and RVH which is in agreement with our previous study where we observed decreased RVP, RVH, and improved RV functions in MCT-induced PH rats treated with ISO [15].

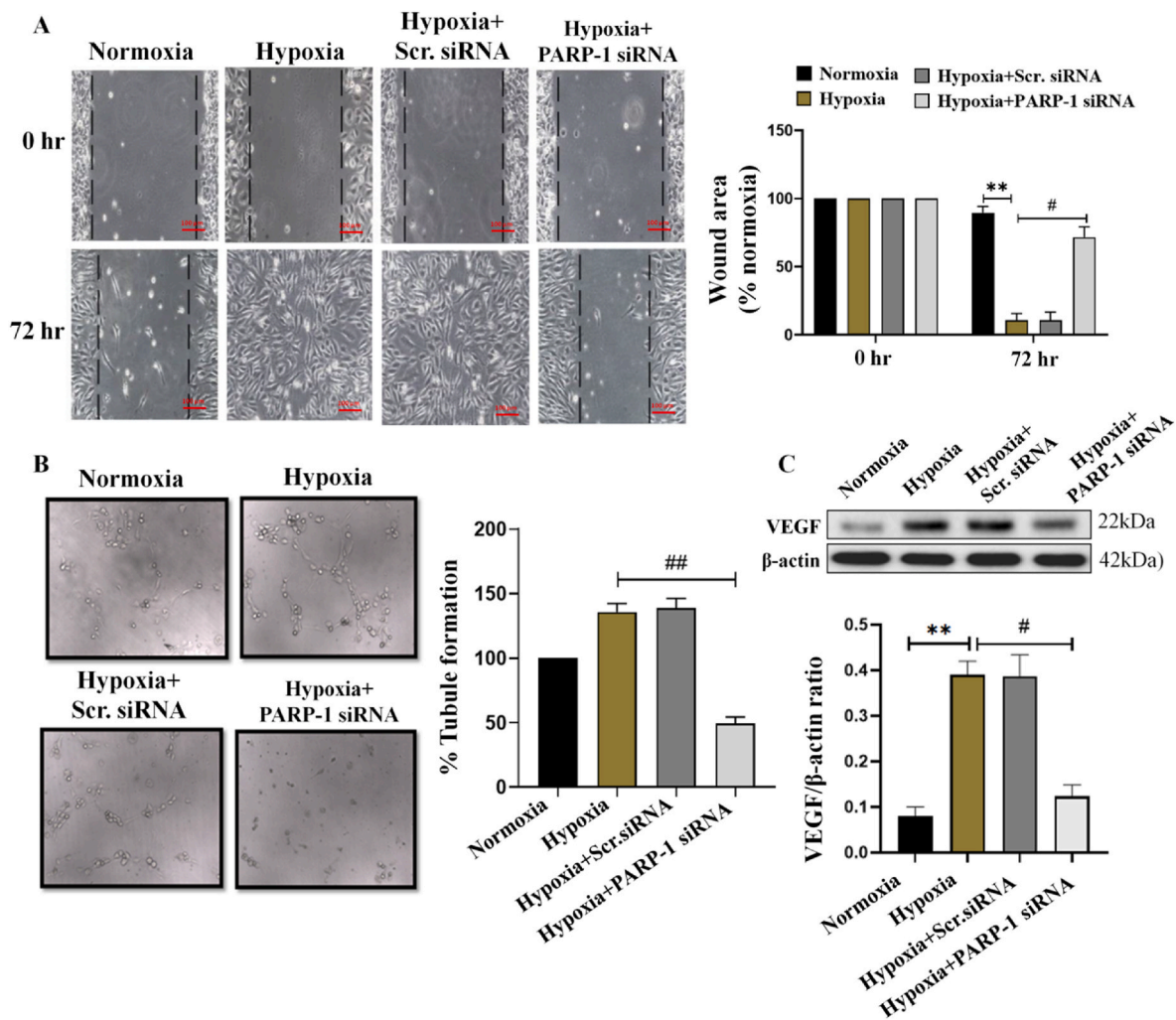


**Fig. 4.** PARP-1 siRNA treatment decreases tubule formation by lowering cell migration and VEGF expression. (A) Representative image of cell migration (Scale bar = 100  $\mu$ m) and analysis of cell migration (% of normoxic control), (B) Representative image of tubule formation (Scale bar = 50  $\mu$ m) and Tubule length (% of normoxic control), and (C) Expression of VEGF in HPAEC after hypoxia exposure. Data are shown as Mean  $\pm$  SEM (n = 3), \*Normoxia vs. Hypoxia, #Hypoxia vs. PARP-1 siRNA. \*\*P < 0.01, ##P < 0.01, and #p < 0.05.

### 3.7. Inhibition of PARP-1 improves endothelial function in MCT-treated rats

Endothelial dysfunction and vascular tone abnormality are crucial factors in the pathogenesis of PH [3]. Previously; various studies have reported reduced pulmonary vascular reactivity in MCT-treated rats [17]. In our study, a significantly decreased vasorelaxation was observed in pulmonary vessels of MCT-treated rats, pre-constricted with phenylephrine. However, pharmacological inhibition of PARP-1 by ISO significantly increased vasorelaxation of pulmonary vessels, hence showing improvement in endothelial functions (Fig. 7A). Moreover, expression of p-eNOS was reduced in the distal pulmonary arteries of MCT-treated rats and was significantly increased by the inhibition of PARP-1, suggesting the involvement of PARP-1 in maintaining endothelial functions in pulmonary vasculature (Fig. 7B).

## 4. Discussion

DNA-damage response (DDR) dysregulation has been identified as a potential risk factor in the pathogenesis of PH [4]. Earlier reports suggest that DNA damage acts as a disease driver and there is increased DNA damage in PAECs and blood cells of PAH patients [24,25]. However, no study as of now has linked the DNA damage and involvement of PARP-1

in endothelial dysfunction associated with PH. Therefore, the present study was designed to understand the involvement of PARP-1 in pulmonary endothelial dysfunction. In our study, we observed increased expression and activity of PARP-1 in hypoxic HPAECs and MCT-treated rats which were significantly decreased by the inhibition of PARP-1. Furthermore, our study also demonstrated that siRNA-mediated PARP-1 silencing attenuated pro-proliferative, pro-inflammatory, and apoptosis resistance phenotypes as well as improved mitochondrial functions and energy balance in hypoxic HPAECs. Moreover, in rats treated with MCT, a PARP-1 inhibitor, ISO, improved pulmonary endothelial function by enhancing vascular reactivity as well as eNOS expression.

DNA damage is elevated in the lungs, distal pulmonary arteries, and PSMCs of PH patients, as well as in rat models of PH [4]. Furthermore, mild to moderate DNA damage, as it occurs in the pulmonary vasculature, activates PARP-1, which causes the recruitment of repair factors at the DNA damage site, allowing PSMCs to survive and potentially proliferate [10]. In corroboration with this, we observed that hypoxia increased PARP-1 expression and activity in HPAECs, which was significantly decreased upon inhibition of PARP-1. Furthermore, our study also demonstrated the increased proliferation of hypoxic HPAECs as evidenced by an increase in cell count as well as protein and mRNA expressions of PCNA and Chk1, respectively. In HPAECs, we also

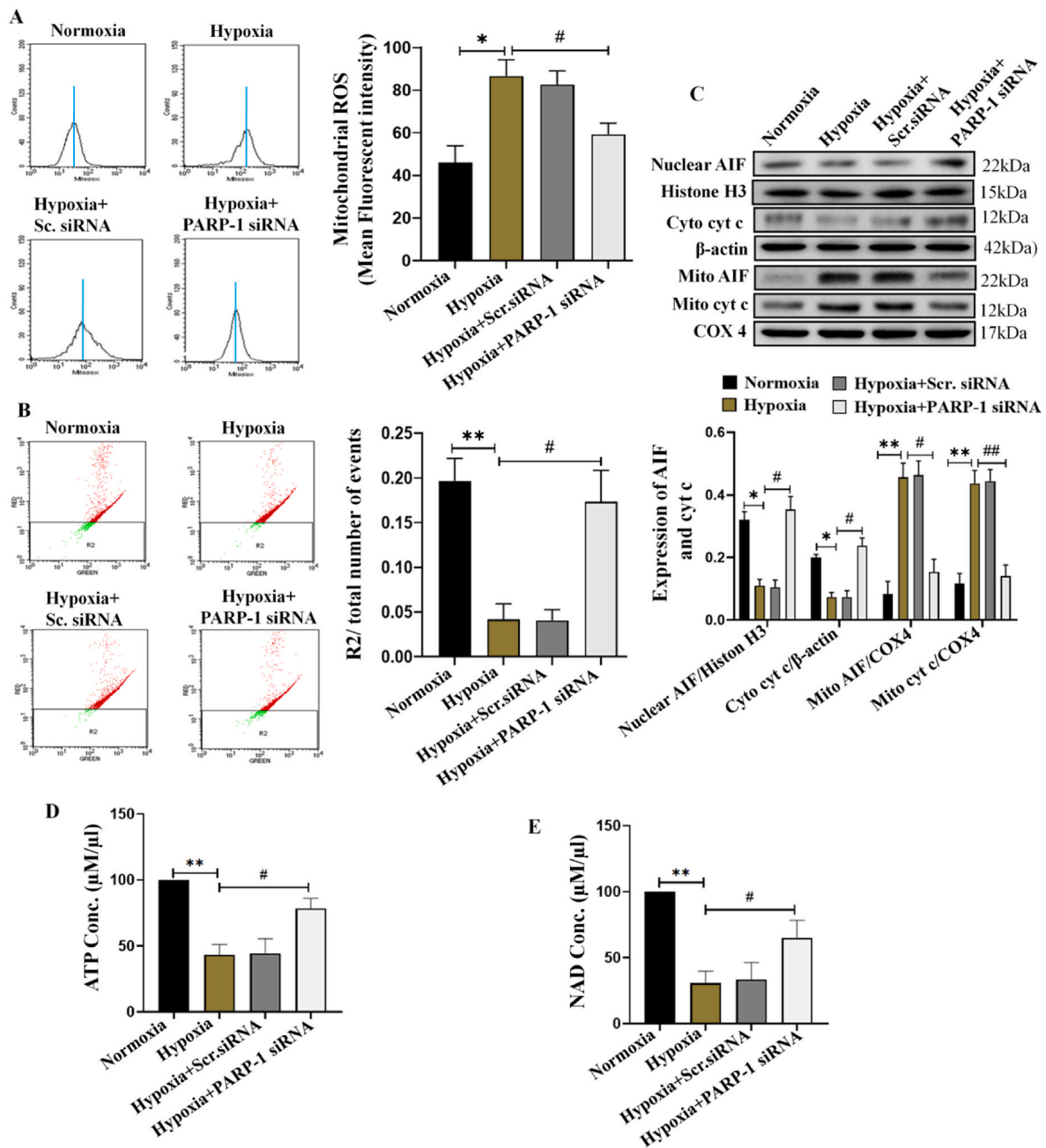


**Fig. 5.** Inhibition of PARP-1 prevents mitochondrial dysfunction by decreasing mROS, preserving MMP as well as improving ATP and NAD levels in hypoxic HPAECs. (A) Determination of mROS and its quantification using Mitosox by FACS (B) MMP and its quantification was determined using JC-1 (MMP probe) by FACS, (C) expressions of Nuclear AIF, cytolitic c, Mito AIF and Mito cyt c in hypoxic HPAECs (D) ATP levels and (E) NAD levels in hypoxic HPAECs. Data are shown as Mean  $\pm$  SEM (n = 3), \*Normoxia vs. Hypoxia, #Hypoxia vs. PARP-1 siRNA. \*\*P < 0.01, \*p < 0.05, ##P < 0.01 and #p < 0.05.

evaluated the expression of cell cycle regulatory proteins and found that hypoxia enhanced the expression of cyclin D1 while decreasing p53 expression. However, upon inhibition of PARP-1 by siRNA, hypoxic HPAECs showed a reduction in proliferation by mitigating all the abnormalities and substantially decreased the expression of cyclin D1 and increased that of p53. These findings are consistent with previous research indicating that PARP-1 inhibition reduces proliferation in PAH-PASMCs by down-regulating miR-204 dependent activation of nuclear factor of activated T cells and hypoxia-inducible factor 1- $\alpha$  [10] and also affects cell cycle and proliferation in various cancers by modulating various signaling pathways such as cellular energetics, death signaling, improper cell cycle regulation, and binding to the inhibitory element of COX-2, which is responsible for mediating

inflammation and activation of VEGF-mediated pro-angiogenesis pathways in cancer cells [26,27].

Inflammation has a key role in the genesis and progression of endothelial dysfunction in PH [3]. Moreover, hypoxia and inflammation are inexorably connected at the cellular, molecular, and clinical levels [28]. In addition, the role of BET proteins BRD2 and BRD4 in the regulation of inflammation has been thoroughly investigated, and their higher levels in HPASMCs and HPAECs subjected to hypoxia were also observed in our previous work [3,17]. As a result, understanding the role of PARP-1 in the regulation of BET proteins was crucial for understanding the PH phenotype. The present study demonstrated that inhibiting PARP-1 lowered the mRNA levels of BRD2 and BRD4 in HPAECs exposed to hypoxia. Furthermore, we also observed higher

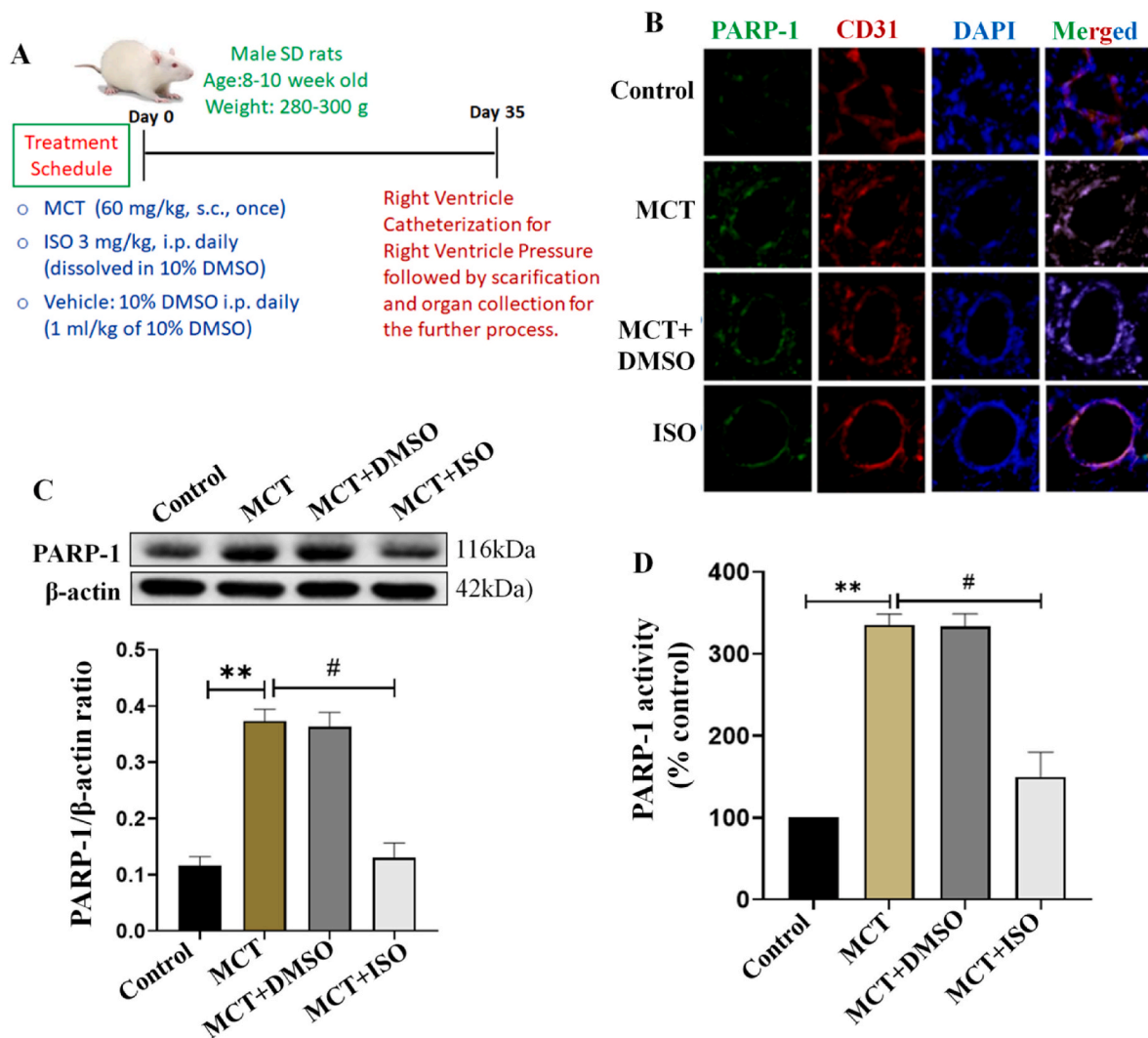


**Fig. 6.** Increased expression and activity of PARP-1 in MCT-treated rats. (A) treatments schedule, (B) PARP-1 expression (Immunofluorescence), (C) PARP-1 expression and quantification (immunoblotting), and (D) PARP-1 activity. Data are shown as Mean  $\pm$  SEM (n = 6), \*Control vs. MCT, #MCT vs. ISO. \*\*P < 0.01 and #p < 0.05.

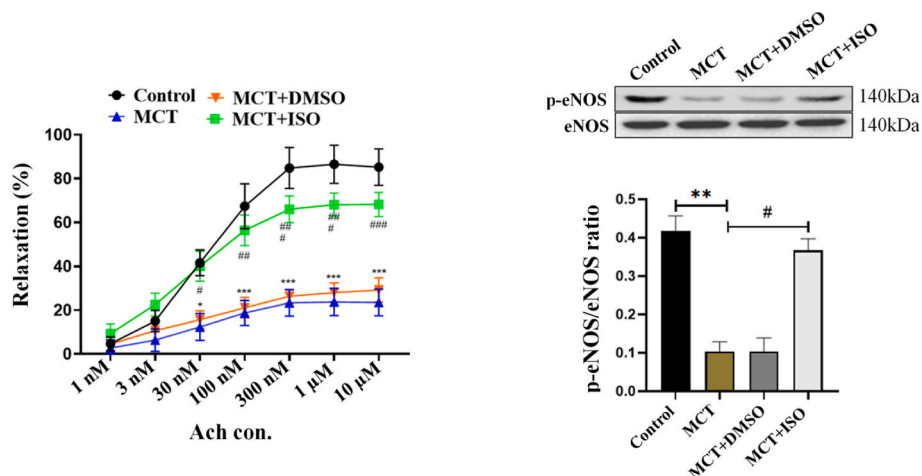


**Fig. 7.** Effect of PARP-1 inhibition preserves endothelial function and increased the expression of p-eNOS in hypoxic HPAECs. (A) Endothelial function in MCT-treated rats and (B) p-eNOS/eNOS expression and quantification. Data are shown as Mean  $\pm$  S.E.M (n = 6), \*Control vs. MCT, #MCT vs. ISO, \*\*\*p < 0.001, \*\*p < 0.01, \*p < 0.05, ###p < 0.01, ##p < 0.01 and #p < 0.05.

levels of the inflammatory cytokines IL-1 $\beta$  and IL-6, as well as a reduction in the anti-inflammatory cytokine IL-10 in hypoxic HPAECs, which were significantly reversed by PARP-1 inhibition. Additionally, the NF- $\kappa$ B expression was also found to be increased in hypoxic HPAECs which was markedly decreased by the inhibition of PARP-1. Previous studies have discussed the role of PARP-1 in various pathophysiological processes such as heart failure, myocardial infarction, atherosclerosis, and neurodegeneration, as well as their contribution to inflammation, which occurs primarily through PARP-1-dependent co-activation in the NF- $\kappa$ B mediated transcription of various inflammatory proteins [29,30]. Furthermore, PARP-1 inhibition or deletion has also been demonstrated to reduce the expression of a host of pro-inflammatory mediators, including cytokines, chemokines, and inducible nitric oxide synthase [31].

In addition, mitochondria govern a range of cellular processes such as ROS generation, proliferation, as well as apoptosis [17]. Alterations in endothelial mitochondrial functions in the pulmonary vasculature are considered to be key regulators of endothelial dysfunction in PH [32]. Furthermore, in PAECs, an increase in mitochondrial membrane potential has been reported in response to hypoxia, which inhibits ATP synthesis and increases mROS production [3]. In accordance with these studies, we observed that mitochondrial membrane potential was hyperpolarized in hypoxic HPAECs, as demonstrated by the decrease in JC-1 monomers relative to normoxia which was effectively reversed by PARP-1 inhibition. Furthermore, we also observed an increased level of mROS in hypoxic HPAECs, which was significantly reduced by PARP-1 siRNA treatment. In addition, PH is associated with altered energy production in the pulmonary vasculature, characterized by the depletion of NAD as well as ATP levels [19] which are similar to the findings obtained in hypoxic HPAECs in this study. However, inhibition of PARP-1 significantly increased the levels of both NAD and ATP, thereby depicting improvement in mitochondrial bioenergetics and consequently mitochondrial functioning. These results are in line with the previous study which demonstrated PARP-1 inhibition preserved mitochondrial structure and mitochondrial genome integrity as well as prevented mitochondrial fragmentation in hypertension-induced myocardial remodeling and stressed cardiomyocytes [33]. Moreover, inhibition of PARP-1 by 3-amino benzamide prevented a reduction in ATP generation in HaCaT cells exposed to the alkylating agent, sulfur mustard, while also influencing the manner of cell death from increasing apoptosis to reducing necrosis [34].

Chronic hypoxia is responsible for the induction of inflammation and the production of ROS and reactive nitrogen species (RNS). These entire variables come together in PH and create a toxic environment for the cells, resulting in DNA damage and, as result PARP-1 activation [15]. Therefore, we aimed to study the effect of PARP-1 inhibition on the regulation of DNA damage in hypoxic HPAECs, using the marker  $\gamma$ -H2AX which was found to significantly increase in hypoxia than in normoxia. Furthermore, an alkaline comet assay designed to detect DNA single-strand and double-strand breaks was performed to measure the extent of DNA damage in hypoxic HPAECs, which revealed considerably enhanced DNA damage as shown by the increased percentage of tail DNA as compared to normoxia. Inhibiting PARP-1 using siRNA, on the other hand, slowed the DNA repair process and increased DNA damage. Accordingly, there are evidences that inhibiting PARP-1 in cells exposed to DNA-damaging agents decreases DNA repair and induces apoptosis while at the same time decreasing necrotic cell death [35]. More importantly, it is interesting to note that PARP-1 inhibitors are more effective against proliferating cells (cancer cells) than against normal cells [35]. Therefore, it can be concluded from the discussion that PARP-1 inhibition may induce apoptotic cell death in hypoxic HPAECs without damaging the normal cells.

In addition, chronic hypoxia exposure promotes apoptosis-resistance phenotype in HPAECs [3]. Studies have reported that mitochondrial hyperpolarization decreases the releases of cytochrome c from mitochondria to cytosol, thus causing apoptosis resistance [17,36]. In line with this, we

found that hypoxia enhanced the mitochondrial membrane potential in HPAECs while lowering the release of cytochrome c from mitochondria to the cytosol. Inhibiting PARP-1, on the other hand, restored mitochondrial membrane potential and enhanced cytochrome c release from mitochondria to cytosol, required for caspase-dependent apoptosis. Furthermore, PARP-1 inhibition increased AIF release from mitochondria to the nucleus, initiating caspase-independent apoptosis. These findings showed that inhibiting PARP-1 caused apoptotic cell death via both caspase-dependent and caspase-independent mechanisms. Moreover, these results were also supported by the decreased expression and activity of pro-apoptotic markers, Bax and caspase 3, respectively, and increased expression of anti-apoptotic protein, Bcl2 in HPAECs exposed to hypoxia which was ameliorated by siRNA mediated PARP-1 inhibition. These findings are in sync with earlier studies which show PARP-1 inhibition induces apoptosis in PAH-PASMCs as well as cancer cells, such as HeLa cells [10,37]. Furthermore, when coupled with cisplatin or temozolomide, the PARP-1 inhibitor PJ34 has been shown to protect mitochondria and induce DNA damage-mediated apoptosis in B16F10 melanoma cells [38].

In addition, angiogenesis is the hallmark of endothelial dysfunction in various diseases including PH. Studies have revealed that PARP-1 regulates cell migration and tubule formation in a number of cells, including endothelial cells, both of which are implicated in neoangiogenesis [16,39]. Similarly in our study, we observed increased cell migration in hypoxic HPAECs which was significantly reduced by the inhibition of PARP-1. Furthermore, since endothelial cell migration is required for neovascularization, we assessed the percentage of tubule formation for *in vitro* determination of neoangiogenesis and found that it was much greater in hypoxic HPAECs compared to normoxic cells. Moreover, we also observed the increased expression of VEGF, a key mediator of angiogenesis, in hypoxic HPAECs. Inhibition of PARP-1 by siRNA significantly decreased the percentage of tubule formation as well as expression of VEGF, demonstrating the improvement of endothelial functions by reducing neoangiogenesis. These studies are in accordance with previous observations which reported that PARP-1 inhibition reduces cell migration, and proliferation and promotes apoptosis of hepatocellular carcinoma cells [39]. Another study revealed that the PARP-1 inhibitor GPI 15427 inhibits VEGF-induced endothelial cell migration, decreases tubule formation, and hence lowers neoangiogenesis, which was also verified in PARP-1 knockout mice that displayed a deficit in growth factor-induced angiogenesis [16].

Furthermore, we employed a rat model of PH induced by a single subcutaneous administration of monocrotaline (MCT) for 35 days to confirm the results obtained *in vitro*. The severity of the condition and survival in PH are strongly related to RVP and hypertrophy and RV dysfunction leading to RHF, a major cause of death in PH [40,41]. As observed in our previous study [15], we found a significant increase in RVP and RVH in rats treated with MCT compared to the control, thus indicating the development of PH. ISO, a PARP-1 inhibitor, significantly reduced both the RVP and the RVH. Furthermore, we also studied the expression and activity of PARP-1 in the pulmonary vascular endothelium of MCT-treated rats and found it increased, which was significantly reduced by the inhibition of PARP-1.

The endothelium plays a vital role in maintaining the vascular tone. Endothelial dysfunction is another important feature of PH that has been reported in various previous studies [3,42,43]. Moreover, the endothelium regulates eNOS, which helps to maintain vascular tone. However, according to several studies including ours, MCT-treated rats exhibit lower pulmonary vascular reactivity [17,44]. In keeping with previous findings, we observed a decrease in acetylcholine-induced vasorelaxation in isolated pulmonary arteries of MCT-treated rats. PARP-1 inhibition restored relaxation and enhanced endothelial functioning. More importantly, PARP-1 inhibition not only improved vascular tone in isolated pulmonary arteries but also increased p-eNOS expression. Thus, it can be argued that PARP-1 inhibition avoided endothelial dysfunction by increasing endothelial vascular tone due to an increase in eNOS

expression. Improvement of endothelial functions following PARP-1 inhibition is supported by previous studies also, PARP-1 inhibition was shown to protect age-dependent endothelial dysfunction by regulating nitric oxide bioavailability via inducible nitric oxide synthase [12]. Furthermore, PARP-1 inhibition, both in *in vitro* and *in vivo* studies, has also been found to markedly reduce the oxidized-LDL-induced increase in endogenous Arginase II production resulting in enhanced eNOS and nitric oxide generation [45].

In conclusion, the present study demonstrated that PARP-1, in addition to DNA repair, regulates pulmonary arterial endothelial dysfunction associated with PH. As has been previously observed in PASMCs, overexpression of PARP-1 has been demonstrated to promote proliferation, apoptosis resistance, and inflammation in HPAECs. Furthermore, hypoxia-induced PARP-1 overexpression has been shown to promote cell migration and tubulogenesis, which together contribute to neoangiogenesis. Inhibiting PARP-1 using siRNA, on the other hand, prevented all abnormalities, thus preserving the functioning of HPAECs. More notably, the current work established the role of PARP-1 in impaired vascular reactivity and eNOS regulation in MCT-treated rats, which was significantly reversed by the treatment with PARP-1 inhibition. PARP-1 inhibition has already been shown to prevent or reverse the pulmonary smooth muscle cell remodeling and RVH associated with PH as shown in previous studies from Meloche et al. and our group, therefore, the current study presented the missing link by describing the protective role of PARP-1 inhibition in preventing pulmonary artery endothelial dysfunction associated with PH.

### Sources for funding

This work was supported by financial grants from the Council of Scientific and Industrial Research (CSIR), CSIR network programs THUNDER (BSC0102), UNDO (BSC0103), MLP0114, and the Department of Biotechnology, India.

### Author statement

Mohammad Shafiq: Methodology, Investigation, Formal analysis, Writing Original Draft, Writing-Review and Editing, Visualization. Zahid Rasool Lone: Methodology, Investigation, Formal analysis, Writing Original Draft, Writing-Review and Editing, Visualization. Adam Olaitan Abdulkareem: Methodology, Investigation, Formal analysis. Gurpreet Kaur: Methodology, Investigation, Formal analysis. Sai Navya: Methodology, Investigation, Formal analysis. Himalaya Singh: Methodology, Investigation, Formal analysis. Kumaravelu Jagavelu: Conceptualization, validation, Resources. Kashif Hanif: Conceptualization, Supervision, Review and Editing, Project administration, Funding acquisition.

### Declaration of competing interest

None.

### Data availability

Data will be made available on request.

### Acknowledgments

We acknowledge Dr. A.L. Vishwakarma, Division of Sophisticated Analytical Instrument.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.pupt.2023.102200>.

### References

- [1] R. Budhiraja, R.M. Tuder, P.M. Hassoun, Endothelial dysfunction in pulmonary hypertension, *Circulation* 109 (2) (2004) 159–165.
- [2] S. Dhole, et al., Comprehensive review on novel targets and emerging therapeutic modalities for pulmonary arterial Hypertension, *Int. J. Pharm.* 621 (2022), 121792.
- [3] M. Shafiq, et al., Involvement of mitogen-activated protein kinase (MAPK)-activated protein kinase 2 (MK2) in endothelial dysfunction associated with pulmonary hypertension, *Life Sci.* 286 (2021), 120075.
- [4] B. Ranchoux, et al., DNA damage and pulmonary hypertension, *Int. J. Mol. Sci.* 17 (6) (2016).
- [5] M. Shafiq, et al., Pyrroloquinoline quinone (PQQ) improves pulmonary hypertension by regulating mitochondrial and metabolic functions, *Pulm. Pharmacol. Ther.* 76 (2022), 102156.
- [6] K. Kurakula, et al., Endothelial dysfunction in pulmonary hypertension: cause or consequence? *Biomedicines* 9 (1) (2021).
- [7] B. Ranchoux, et al., Endothelial dysfunction in pulmonary arterial hypertension: an evolving landscape (2017 Grover Conference Series), *Pulm. Circ.* 8 (1) (2018), 2045893217752912.
- [8] W. Xu, S.C. Erzurum, Endothelial cell energy metabolism, proliferation, and apoptosis in pulmonary hypertension, *Compr. Physiol.* 1 (1) (2011) 357–372.
- [9] M.T. Mathews, B.C. Berk, PARP-1 inhibition prevents oxidative and nitrosative stress-induced endothelial cell death via transactivation of the VEGF receptor 2, *Arterioscler. Thromb. Vasc. Biol.* 28 (4) (2008) 711–717.
- [10] J. Meloche, et al., Role for DNA damage signaling in pulmonary arterial hypertension, *Circulation* 129 (7) (2014) 786–797.
- [11] Y. Wang, W. Luo, PARP-1 and its associated nucleases in DNA damage response, *DNA Repair* 81 (2019), 102651.
- [12] G.H. Zhang, et al., Poly(ADP-ribose)polymerase 1 inhibition protects against age-dependent endothelial dysfunction, *Clin. Exp. Pharmacol. Physiol.* 42 (12) (2015) 1266–1274.
- [13] J. Chiang, et al., Honokiol protects rats against eccentric exercise-induced skeletal muscle damage by inhibiting NF-kappaB induced oxidative stress and inflammation, *Eur. J. Pharmacol.* 610 (1–3) (2009) 119–127.
- [14] C. Gebhard, et al., Poly(ADP-ribose) polymerase-1 protects from oxidative stress induced endothelial dysfunction, *Biochem. Biophys. Res. Commun.* 414 (4) (2011) 641–646.
- [15] G. Kaur, et al., Poly (ADP-ribose) polymerase-1: an emerging target in right ventricle dysfunction associated with pulmonary hypertension, *Pulm. Pharmacol. Ther.* 30 (2015) 66–79.
- [16] L. Tentori, et al., Poly(ADP-ribose) polymerase (PARP) inhibition or PARP-1 gene deletion reduces angiogenesis, *Eur. J. Cancer* 43 (14) (2007) 2124–2133.
- [17] M. Shafiq, et al., Inhibition of mitogen-activated protein kinase (MAPK)-Activated protein kinase 2 (MK2) is protective in pulmonary hypertension, *Hypertension* 77 (4) (2021) 1248–1259.
- [18] S. Swarnkar, et al., Astrocyte activation: a key step in rotenone induced cytotoxicity and DNA damage, *Neurochem. Res.* 37 (10) (2012) 2178–2189.
- [19] N. Singh, et al., Inhibition of fatty acid synthase is protective in pulmonary hypertension, *Br. J. Pharmacol.* 173 (12) (2016) 2030–2045.
- [20] N.V. Iyer, et al., Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha, *Genes Dev.* 12 (2) (1998) 149–162.
- [21] K.M. Porter, et al., Chronic hypoxia promotes pulmonary artery endothelial cell proliferation through H2O2-induced 5-lipoxygenase, *PLoS One* 9 (6) (2014), e98532.
- [22] S. Song, et al., Hypoxia inhibits pulmonary artery endothelial cell apoptosis via the e-selectin/biliverdin reductase pathway, *Microvasc. Res.* 106 (2016) 44–56.
- [23] B.L. Krock, N. Skuli, M.C. Simon, Hypoxia-induced angiogenesis: good and evil, *Genes Cancer* 2 (12) (2011) 1117–1133.
- [24] S. Sharma, M.A. Aldred, DNA damage and repair in pulmonary arterial hypertension, *Genes* 11 (10) (2020).
- [25] C. Federici, et al., Increased mutagen sensitivity and DNA damage in pulmonary arterial hypertension, *Am. J. Respir. Crit. Care Med.* 192 (2) (2015) 219–228.
- [26] L. Wang, et al., PARP1 in carcinomas and PARP1 inhibitors as antineoplastic drugs, *Int. J. Mol. Sci.* 18 (10) (2017).
- [27] A.N. Weaver, E.S. Yang, Beyond DNA repair: additional functions of PARP-1 in cancer, *Front. Oncol.* 3 (2013) 290.
- [28] H.K. Eltzschig, P. Carmeliet, Hypoxia and inflammation, *N. Engl. J. Med.* 364 (7) (2011) 656–665.
- [29] A. Mangerich, A. Burkle, Pleiotropic cellular functions of PARP1 in longevity and aging: genome maintenance meets inflammation, 2012, *Oxid. Med. Cell. Longev.* (2012), 321653.
- [30] S. Pazzaglia, C. Pioli, Multifaceted role of PARP-1 in DNA repair and inflammation: pathological and therapeutic implications in cancer and non-cancer diseases, *Cells* 9 (1) (2019).
- [31] P. Pacher, C. Szabo, Role of poly(ADP-ribose) polymerase 1 (PARP-1) in cardiovascular diseases: the therapeutic potential of PARP inhibitors, *Cardiovasc. Drug Rev.* 25 (3) (2007) 235–260.
- [32] Q. Yu, S.Y. Chan, Mitochondrial and metabolic drivers of pulmonary vascular endothelial dysfunction in pulmonary hypertension, *Adv. Exp. Med. Biol.* 967 (2017) 373–383.
- [33] K. Ordog, et al., Mitochondrial protective effects of PARP-inhibition in hypertension-induced myocardial remodeling and in stressed cardiomyocytes, *Life Sci.* 268 (2021), 118936.

- [34] K. Kehe, et al., Inhibition of poly(ADP-ribose) polymerase (PARP) influences the mode of sulfur mustard (SM)-induced cell death in HaCaT cells, *Arch. Toxicol.* 82 (7) (2008) 461–470.
- [35] V. Cepeda, et al., Poly(ADP-ribose) polymerase-1 (PARP-1) inhibitors in cancer chemotherapy, *Recent Pat. Anti-Cancer Drug Discov.* 1 (1) (2006) 39–53.
- [36] J. Cai, J. Yang, D.P. Jones, Mitochondrial control of apoptosis: the role of cytochrome c, *Biochim. Biophys. Acta* 1366 (1–2) (1998) 139–149.
- [37] U. Ghosh, N.P. Bhattacharyya, Induction of apoptosis by the inhibitors of poly (ADP-ribose)polymerase in HeLa cells, *Mol. Cell. Biochem.* 320 (1–2) (2009) 15–23.
- [38] A.M. Cseh, et al., PARP inhibitor PJ34 protects mitochondria and induces DNA-damage mediated apoptosis in combination with cisplatin or temozolomide in B16F10 melanoma cells, *Front. Physiol.* 10 (2019) 538.
- [39] X. Mao, et al., Inhibitors of PARP-1 exert inhibitory effects on the biological characteristics of hepatocellular carcinoma cells in vitro, *Mol. Med. Rep.* 16 (1) (2017) 208–214.
- [40] J.J. Ryan, et al., Right ventricular adaptation and failure in pulmonary arterial hypertension, *Can. J. Cardiol.* 31 (4) (2015) 391–406.
- [41] N. Singh, et al., Involvement of fatty acid synthase in right ventricle dysfunction in pulmonary hypertension, *Exp. Cell Res.* 383 (2) (2019), 111569.
- [42] N. Singh, et al., Fatty acid synthase modulates proliferation, metabolic functions and angiogenesis in hypoxic pulmonary artery endothelial cells, *Eur. J. Pharmacol.* 815 (2017) 462–469.
- [43] K.H. Park, W.J. Park, Endothelial dysfunction: clinical implications in cardiovascular disease and therapeutic approaches, *J. Kor. Med. Sci.* 30 (9) (2015) 1213–1225.
- [44] H. Christou, et al., Improved pulmonary vascular reactivity and decreased hypertrophic remodeling during nonhypercapnic acidosis in experimental pulmonary hypertension, *Am. J. Physiol. Lung Cell Mol. Physiol.* 302 (9) (2012) L875–L890.
- [45] Q. Wang, et al., Poly (ADP-Ribose) polymerase 1 mediated Arginase II activation is responsible for oxidized LDL-induced endothelial dysfunction, *Front. Pharmacol.* 9 (2018) 882.