Quercetin, Perillyl Alcohol, and Berberine Ameliorate Right Ventricular Disorders in Experimental Pulmonary Arterial Hypertension: Effects on miR-204, miR-27a, Fibrotic, Apoptotic, and Inflammatory Factors

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Abstract: Pulmonary arterial hypertension (PAH) is a pulmonary vascular disease causing right ventricular (RV) hypertrophy, failure, and death. Some miRNAs are involved in the pathophysiology of PAH. As the current treatments cannot prevent the progression of the disease, we investigated whether 3 plant derivatives, namely perillyl alcohol (PA), quercetin (QS), and berberine (BBR), can improve RV function and affect the expression of miR-204, miR-27a, and biochemical factors in monocrotaline-induced PAH (MCT-PAH). Thirty-six rats were divided into control (CTL), MCT, MCT+Veh (vehicle), MCT+PA, MCT+QS, and MCT + BBR groups (n = 6 each). After inducing PAH using MCT (60 mg/kg), PA (50 mg/kg), QS (30 mg/kg), and BBR (30 mg/kg) were administrated daily for 3 weeks. miR-204 expression, total antioxidant capacity, and antiapoptotic protein Bcl-2 significantly declined in the RV of PAH rats, and PA, QS, and BBR treatment significantly compensated for these decreases. Proapoptotic protein Bax and p21 cell cycle inhibitor increased in the RV. All 3 herbal derivatives compensated for Bax increase, and BBR caused a decrease in p21. TNFα, IL-6, and malondialdehyde increased in the RV, and PA, QS, and BBR significantly counterbalanced these increases. miR-27a expression was not affected by MCT and plant derivatives. Overall, PA, QS, and BBR improved ventricular disorders in rats with PAH by decreasing inflammation, apoptosis, and fibrosis and increasing the antioxidant-to-oxidant ratio. Therefore, these herbal derivatives may be considered as target therapeutic goals for this disease either alone or in combination with current medications.

Key Words: pulmonary arterial hypertension, perillyl alcohol, quercetin, berberine, apoptosis, inflammation, oxidative stress

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INTRODUCTION

Pulmonary arterial hypertension (PAH) is a pulmonary vascular disease identified by chronic vascular stenosis and the occlusion of arterioles and small arteries caused by vascular remodeling.[1] One pathologic process leading to vascular stenosis is the increase in cellular proliferation in vascular walls and the decrease in their apoptosis. Vascular stenosis causes hypertension in pulmonary arteries, and subsequently, this imposes an afterload on the right ventricle. Increase in afterload at first results in the adaptive hypertrophy of the right ventricle; however, with the progression of the disease, the ventricle becomes dilated and its contraction becomes weaker and deficient. Ventricular perfusion defect, angiogenesis, inflammation, changes in autonomic nervous signaling, and fibrosis are involved in the progression of maladaptive hypertrophy and fibrillation of the right ventricle (RV).[1,2] RV dysfunction can lead to death if left untreated.[3] Because no quite effective medication has been found to treat this disease, it is crucial to try to find medications that improve cardiac (and pulmonary) disorders in pulmonary arterial hypertension.

One of the pathologic factors in the RV is inflammation, which can be triggered by local and systemic stimuli. RV inflammation impairs the contractibility of the right ventricle, leads to maladaptive remodeling, and creates a vicious circle between RV and pulmonary vascular defects leading to an increase in vascular remodeling.[2] PAH cause RV inflammation contractile dysfunction and hypertrophy, and IL-6, TNFα, and oxidative stress are involved in this phenomenon.[2,4–6] Excessive afterload-induced cardiac hypertrophy leads to extracellular matrix deposition, cardiac fibroblasts proliferation, and myocyte hypertrophy, and TGFβ1

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plays the key role.[7] TGFβ1 expression increases in the hypertrophied myocardium, and its expression in the human heart has been associated with fibrosis caused by pressure loading.[8]

MicroRNAs are a large family of small noncoding RNAs (containing roughly 22 nucleotides). In the miRBase 20.0 database, more than 2500 microRNAs are reported to exist in humans.[9] Dysregulation of microRNAs occurs in the lung tissue in PAH and in the right ventricle.[1] miR-204 is one of the most important microRNAs in PAH that decreases in the lung and in the heart.[10] In ventricular remodeling due to MCT-induced PAH, it was revealed that the expression of miR-204 has significantly changed[11] and miR-204 has increased vascular remodeling with effects on inducible factor 1a (HIF1a) and nuclear factor of activated T-cells c2 (NFATc2) as main targets in this pathway.[12] Contrarily, it was indicated that in congestive heart failure, miR-27a expression increases in cardiomyocytes, and miR-27a amendment is associated with a decline in collagen deposition in the myocardium of this HF model and improved cardiac function.[13] However, the role of this microRNA in heart complications caused by PAH has not been investigated.

Nowadays, active plant components have attracted the attention of researchers hoping to benefit from them in treating incurable diseases. Quercetin (QS), berberine (BBR), and perillyl alcohol (PA) are among these plant compounds that share anti-inflammatory, antioxidative, and antiproliferative effects.[14–16] These active ingredients are present in the human diet; and therefore, they are compatible to the body. QS is a flavonoid found in large amounts in onion, asparagus, apple, and different kinds of berries.[17] BBR is a monoterpene found in barberry and plants such as rhizoma coptidis.[18] PA is an alkaloid in tropical fruits, as well as in cherry, ginger, and blueberry.[19]

In the previous study, we showed that PA and QS significantly decreased pulmonary complications due to experimental PAH and reduced pulmonary vascular remodeling by affecting microRNA-204 and its target genes.[20] In this study, we investigated the effectiveness of these 2 active ingredients and BBR on cardiac disorders resulting from PAH to determine whether the improvement seen in the lung can be observed in cardiac complications of the disease as well. The probable mechanism that may mediate the ameliorating effect of these compounds was also investigated.

METHODS

The study was approved by the Ethics Committee of the Kerman University of Medical Sciences with the ethics approval code, IR.KMU.REC.1395.244. Thirty-six male Wistar rats weighing from 220 to 280 g were used. The animals were kept in the animal house under conditions of a 12-hour/12-hour light/dark cycle and the temperature of 23 ± 2°C. According to the results of our previous study,[20] the optimum dose of PA, QS, and BBR in ameliorating complications of PAH in the lungs was 50 mg/kg, 30 mg/kg, and 30 mg/kg, respectively. The rats were divided randomly into 6 groups (n = 6 each), including control (CTL), MCT, MCT + Veh, MCT + PA, MCT + QS, and MCT + BBR. If an animal died before the conclusion of the study, another animal would replace it from the beginning of the protocol so that the minimum sample size of 6 would be maintained for all groups by the end of the study. This sample size was found to be sufficient to determine the significance of changes in the level of studied variables among the groups.

Induction of Pulmonary Arterial Hypertension

To induce PAH, 60 mg/kg of monocrotaline in 0.2 mL was subcutaneously administered to the animals on day zero.[20] In the normal control group, 0.2 mL of normal saline was administered. After 3 weeks when the animals had developed the disease,[21] they were injected a daily dose of either vehicle (0.5 mL 5% ethanol) or PA (50 mg/kg), QS (30 mg/kg), or BBR (30 mg/kg), in weeks 4–6.

At the end of week 6 (day 43), the rats were anesthetized by intraperitoneal administration of sodium thiopental (100 mg/kg). After ensuring deep anesthesia, the chest was opened, and the heart was removed. The heart was washed using physiological saline and dried with sterilized gauze. The right ventricle was separated from the rest of the heart using delicate surgical scissors and weighed. The ratio of the RV weight to body weight was calculated as an index of RV hypertrophy.

A part of the right ventricle was frozen at −80°C for molecular studies. Half of the right ventricle was fixed in formaldehyde, embedded in paraffin, and cut into thin sections. The sections were stained with Masson's trichrome to determine the fibrosis level. The severity of fibrosis was scored as follows: 0 = normal, 1 = mild, 2 = moderate, and 3 = severe by a pathologist who was blind to the animal grouping.[22] Each section was observed in 6 areas, and the mean score was calculated.

Immunohistochemistry Staining

Immunohistochemistry staining was used to assess caspase-3 expression in the right ventricle. In this method, after preparing 3-μm sections, the sections were prepared according to the protocol and exposed to the primary rabbit cleaved anticaspase-3 (1:150, Cat. no.: RBK009-05, Zytomed, Germany) for 24 hours. Then, they were washed and exposed to secondary antibodies (Mouse/Rabbit UnoVue Detection System, the Netherland) for 1 hour. Finally, hematoxylin and eosin staining was performed, and the slides were analyzed using a light microscope (Nikon-50i, Japan). Subsequently, the number of active caspase-3-positive cells and their percentage were determined.[23]

Assessment of Inflammation

To assess inflammation in the heart tissue, the amount of TNFα and IL-6 was measured by the ELISA method and with appropriate kits. The tissues of the right ventricle were homogenized in phosphate-buffered saline and centrifuged. The supernatant was collected to measure the inflammatory factors. First, the samples and diluted standard solutions were added to the wells, and then the detection antibody and horseradish peroxidase were added. After incubation at 37°C for 1 hour, the plate was washed using the appropriate solution. Afterward, chromogens A and B were added to the well to react with the enzyme and form a colored solution. Finally, the plate was incubated at 37°C for 10 minutes, the stop

TABLE 1. Sequences of Forward and Reverse Primers Used in Real-Time PCR

miRNA-204	RT	5′-GTTGGCTCTGGTGCAGGGT CCGAGGTATTCGCACCAGAG CCAACAGGCAT-3′	23
	Forward	5′-GCGGCGGTTCCC TTTGTCATCCT-3′	
	Reverse	5′- GTGCAGGGTCCGAGGT-3′	
RNU6	Forward	5′-CTCGCTTCGGCAGCACA-3′	24
	Reverse	5′-AACGCTTCACGAATTTGCGT-3′	
miRNA-27a	RT	5′ GTCGTATCCAGTGC AGGGTCCGAGGTATTCGCAC TGGATACGACGCGGAA 3′	24
	Forward	5′ CGGCGGTTTCACAGTGGCTAAG 3′	
	Reverse	5′ CCAGTGCAGGGTCCGAGGTAT 3′	

solution was added, and absorption was read using an ELISA reader (Eliza MAT 2000, DRG instruments, GmbH) at 450 nanometers.[24]

Measuring Oxidative Stress Indices

The amount of malondialdehyde (MDA), glutathione peroxidase, and catalase, as well as the total antioxidant capacity (TAC), was measured in the aforementioned supernatant. To measure the glutathione peroxidase activity, the Paglia method was used. The samples and the reaction material were mixed, and absorption was measured at 340 nm.[25]

Catalase activity was measured based on Sinha's method, with some modifications. Samples and reaction material were mixed and added to boiling water for 10 minutes. Then, absorption was measured at 570 nm.[26]

The Nalondi lipid peroxidation assay kit (CAT NO: NS-15022 Nalondi) was used to measure MDA. This method provides a simple, repeatable, and standard way of measuring the amount of MDA in biological samples.[26] In short, MDA reacts with thiobarbituric acid at a high temperature, and a pink-colored mixture with absorption at 540 nm is formed.

To measure TAC, Naxifer assay kit (CAT NO: NS-15012 Naxifer) was used. This kit measures the antioxidant capacity of biomolecules in different samples based on the ferric reducing antioxidant power using a single-electron transfer mechanism. Discoloration due to reaction at 593 nm is measured, and the resulting graph and standard are used to calculate the antioxidant capacity.[27]

Measurement of miR-204 and miR-27a Expression

To assess the expression of miR-204 and miR-27a in the right ventricle, real-time polymerase chain reaction was used. At first, to extract the total RNA, tissue samples were homogenized using miRNA extractor solution and total RNA was extracted based on the protocol of the total RNA Mini-Prep Kit (Bio Basic, Canada). The concentration and purity of the extracted RNA were assessed using a NanoDrop (ND-2100, Thermo Fisher Scientific, Waltham, MA). In the next stage, cDNA synthesis was performed for miRNA-204 and miRNA-27a using PrimeScript first cDNA Synthesis Kit (Takara Bio, Japan) and RT-specific primer. In the real-time stage, miRNA expressions were assessed using Amplicon master mix and miRNA-specific stem-loop primer using StepOnePlus system (Applied Biosystem, Waltham, MA). The internal control in both miRNAs was U6. The primer sequences for miR-204 and miR-27a are presented in Table 1.

When the reaction was finished in the real-time device, the measured CTs of each gene were analyzed in Excel. A fold change in the expression of genes and miRNAs compared with their control genes was calculated based on the following formula:

$$\text{Fold change} = 2^{-\Delta\Delta CT}$$

where ΔΔCT is the difference between the ΔCT of each group and the control group. ΔCT equals the difference between CT gene and its internal control CT.

$$\Delta\Delta CT = \left[(\text{CT gene} - \text{CT RNu6})_{\text{treatment}} - (\text{CT gene} - \text{CT RNu6})_{\text{CTL}}\right]$$

where RNu6 = internal control

Western Blot

The expression of Bax, Bcl-2, and p21 proteins was measured in the right ventricle using western blot. Tissue samples were homogenized in ice-cold RIPA buffer containing a protease inhibitor and subsequently centrifuged for 15 minutes at 12,000 rpm. Total protein concentration in the supernatant was measured using the Bradford protein assay. The same amounts of protein were electrophoretically run on sodium dodecyl sulfate-PAGE gel and then transferred onto a nitrocellulose membrane. After blocking overnight at 4°C, the membranes were incubated with anti-Bax (1/10000, Cat No: ab32503, Abcam, Cambridge, MA), anti-Bcl-2 (1/1000, Cat No: ab196495, Abcam), anti-p21 (1/2000, Cat No: ab109199, Abcam), and anti-beta actin-loading control antibodies (1/2500, Cat No: ab8227; Abcam) for 3 hours at room temperature. Then, membranes were washed 3 times with TBST and

incubated with goat anti-rabbit IgG H&L (horseradish peroxidase) secondary antibodies (1/10000, Cat No: ab6721; Abcam). β-actin was used as the internal control.

Statistical Analysis

Data were expressed as mean ± SEM. SPSS 16 was used for statistical analysis. First, the normal distribution of data was checked by the Shapiro–Wilk test. If the distribution was normal, one-way analysis of variance was used for comparison among the groups, followed by Tukey's post hoc test in case of significance. If the distribution was not normal, the Kruskal–Wallis test was used. P values of 0.05 or less were considered statistically significant.

RESULTS

Fibrosis in the Right Ventricle

The level of fibrosis in RV is presented in Figure 1. PAH significantly increased the level of fibrosis ($P < 0.001$). Treatment with PA, QS, and BBR significantly reduced the level of fibrosis ($P < 0.001$ for all 3 cases). However, the effectiveness of BBR was higher than the other 2.

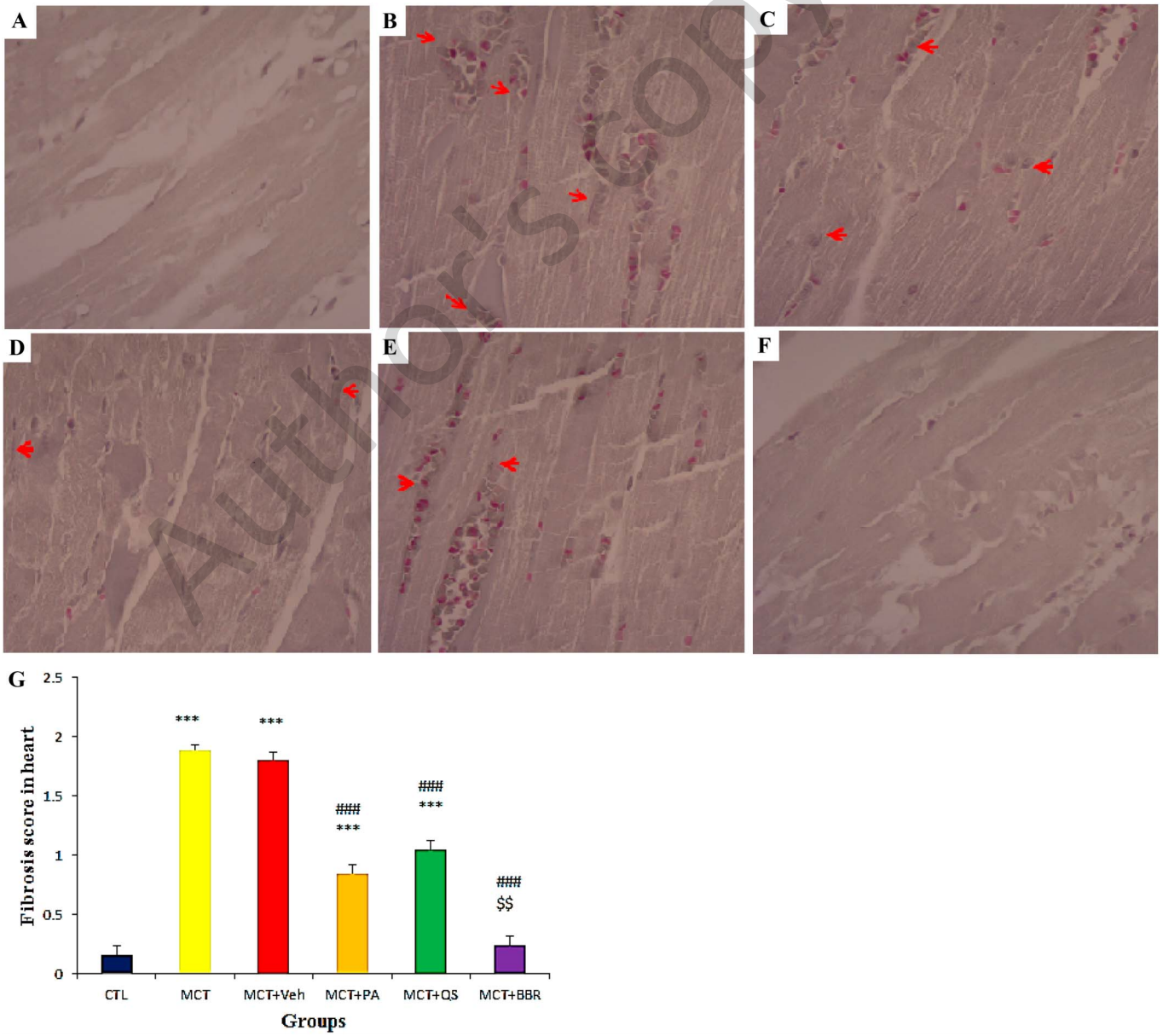

FIGURE 1. Pathological images showing fibrosis (red arrowheads) in the RVs of the studied groups with Masson's trichrome staining. Control group (A), MCT (B), MCT + Veh (C), MCT + QS (D), MCT + PA (E), and MCT + BBR (F), and the relative quantitative data in the groups (G). ***$P < 0.001$ versus the control group, ###$P < 0.001$ versus the MCT + Veh, and $$$P < 0.01$ versus the MCT + PA group. The number of animals in each group is 6 (n = 6) and the magnification, ×100.

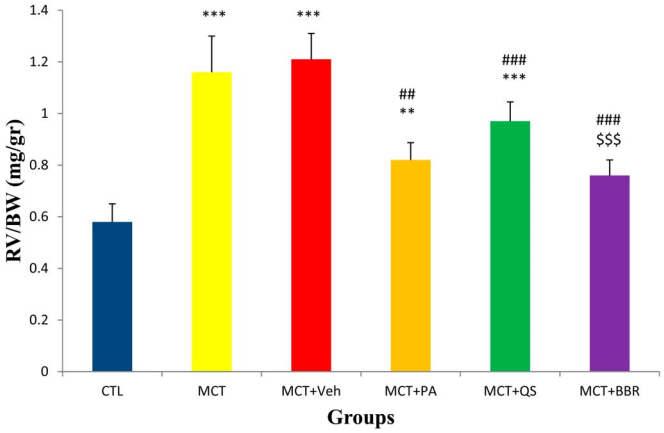

FIGURE 2. RV-to-body weight ratio as RV hypertrophy index in the study groups. n = 6, ***$P < 0.001$ versus the control group, ###$P < 0.001$ versus the MCT + Veh group, and \$\$\$$P < 0.001$ versus MCT + QS.

Hypertrophy of the Right Ventricle

The RV weight-to-body weight ratio in studied groups is indicated in Figure 2. PAH increased the hypertrophy index of the right ventricle ($P < 0.001$). Treatment with all 3 plant derivatives significantly decreased hypertrophy compared with the MCT+Veh group ($P < 0.001$ for all 3 cases). BBR reduced the fibrosis to the control level.

miRNA-204 and miRNA-27a Expression and Response to Treatment

miR-204 significantly decreased in MCT groups compared with the control group. Treatment with PA, QS, and BBR for 3 weeks almost restored the miR-204 level to control (Fig. 3A). QS was more effective derivative compared with the other 2 ($P < 0.05$). Unlike miR-204, the expression of miR-27a did not show significant changes, and treatment with PA, QS, and BBR has no significant effect on the expression of this miRNA (Fig. 3B).

Inflammatory Factors and Response to Treatment

Monocrotaline increased the expression of IL-6 and TNFα in the RVs of studied groups (Fig. 4). PA, QS, and BBR restored the expression of these inflammatory factors to the control level ($P < 0.001$). There was no significant difference among the effect of 3 derivatives.

Oxidative Stress Factors in RV and Response to Treatment

In the right ventricle of MCT-induced PAH rats, the activity of catalase and glutathione peroxidase antioxidants did not change significantly compared with the control group. However, the total antioxidant capacity decreased, and after 3 weeks of treatment with PA ($P < 0.01$), QS ($P < 0.001$), and BBR ($P < 0.01$), a significant compensatory increase was observed (Fig. 5). MDA as the oxidant indicator increased in the MCT-induced rats ($P < 0.001$). PA reduced this indicator to the control level ($P < 0.05$); however, QS and BBR did not have a significant effect on it.

Moreover, the oxidant-to-antioxidant capacity ratio in the MCT and MCT + Veh groups increased compared with the control group. In all the 3 treatment groups, a significant compensatory decrease was observed in this ratio. The difference between the effects of the 3 derivative was not significant.

Apoptotic Indices in RV and Response to Treatment

The expression of Bax (proapoptotic protein) in the RV of the PAH-induced rats showed significant increase compared with the control group (Fig. 6) ($P < 0.001$). Three weeks of treatment with PA, QS, and BBR restored the expression of this protein to the control level. There was no difference among the effects of the 3 derivatives.

The expression of antiapoptotic protein Bcl-2 in the RV of nontreated rats (MCT and MCT + Veh groups) decreased significantly. Treatment with PA, QS, and BBR significantly compensated the decrease in the level of this protein. Among these, the effect of BBR was complete, and QS and BBR were more effective than PA. The Bcl-2/Bax ratio in the MCT and MCT + Veh groups showed a significant decrease (Fig. 6). All 3 compounds acted to compensate for the mentioned reduction, and their effects were in the order of BBR > QS > PA.

The cell cycle inhibitor p21 protein increased significantly in the MCT and MCT + Veh groups compared with the control group ($P < 0.05$). Among the treatments, PA was ineffective while the other 2 products had a significant compensatory effect on p21.

The expression of the apoptotic factor caspase-3 (assessed by the immunohistochemistry method) showed that cells containing caspase-3 significantly increased in the cardiomyocytes. PA treatment recovered the increase in caspase-3 expression by more than 50%, BBR by about 60%, and QS by more than 95%.

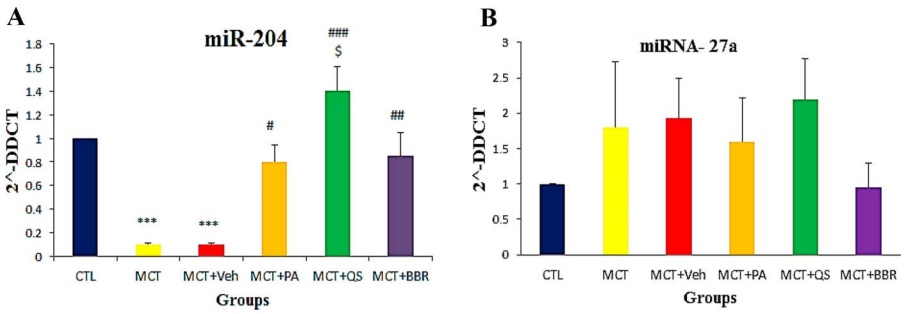

FIGURE 3. Relative expression (mean ± SEM) of miR-204 (A) and miR-27a (B) in the RVs of the study groups. n = 6, ***$P < 0.001$ versus the control group; ###$P < 0.001$, ##$P < 0.01$, and #$P < 0.05$ versus the MCT + Veh group; and \$$P < 0.05$ versus MCT + PA.

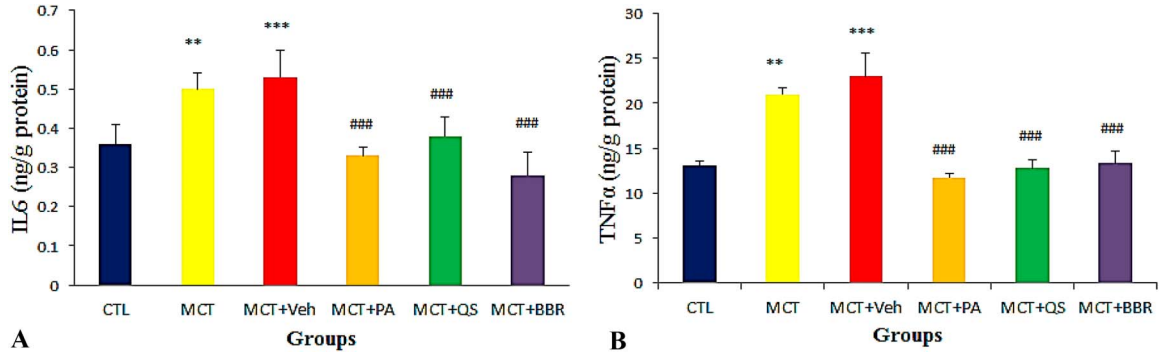

FIGURE 4. Effect of PAH on inflammatory factors in RV. Monocrotaline significantly increased IL-6 (A) and TNF-α (B) levels in RV ($P < 0.001$). Treatment with PA, QS, and BBR for 3 weeks compensated for the increase in these factors. ***$P < 0.001$ and $P < 0.01$ versus the control group and ## $P < 0.01$ versus MCT + Veh. n = 6 in each group.

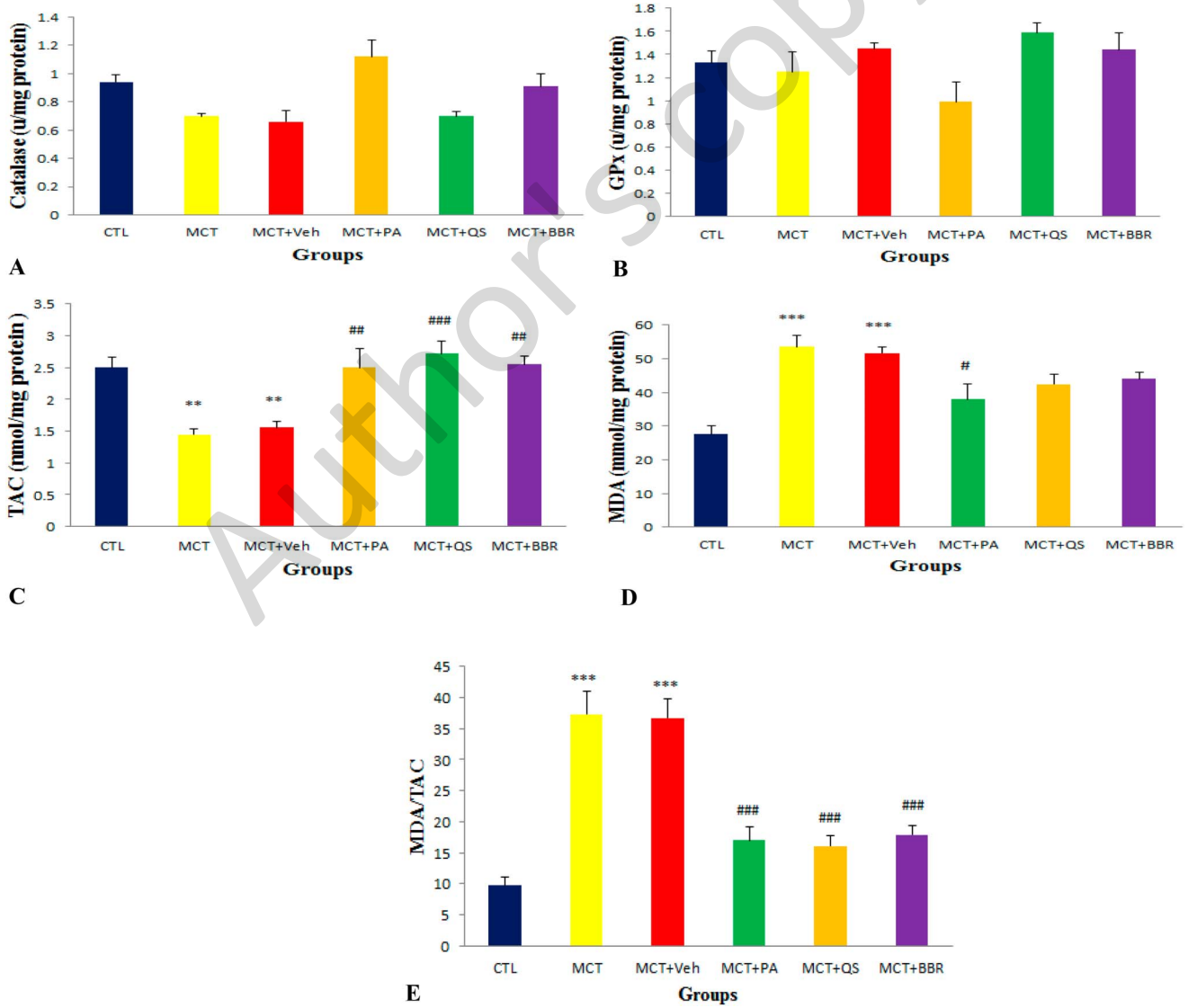

FIGURE 5. PAH effect on the oxidant and antioxidant factors in RV of the studied groups. The antioxidants, catalase (A), glutathione peroxidase (B), total antioxidant capacity (C), MDA oxidant (D), and MDA-to-total antioxidant ratio (E). The data are presented as means ± SEM. ***$P < 0.001$ and **$P < 0.01$ versus the control group and #$P < 0.05$, ##$P < 0.01$, and ###$P < 0.001$ versus the MCT + Veh group. n = 6 in each group.

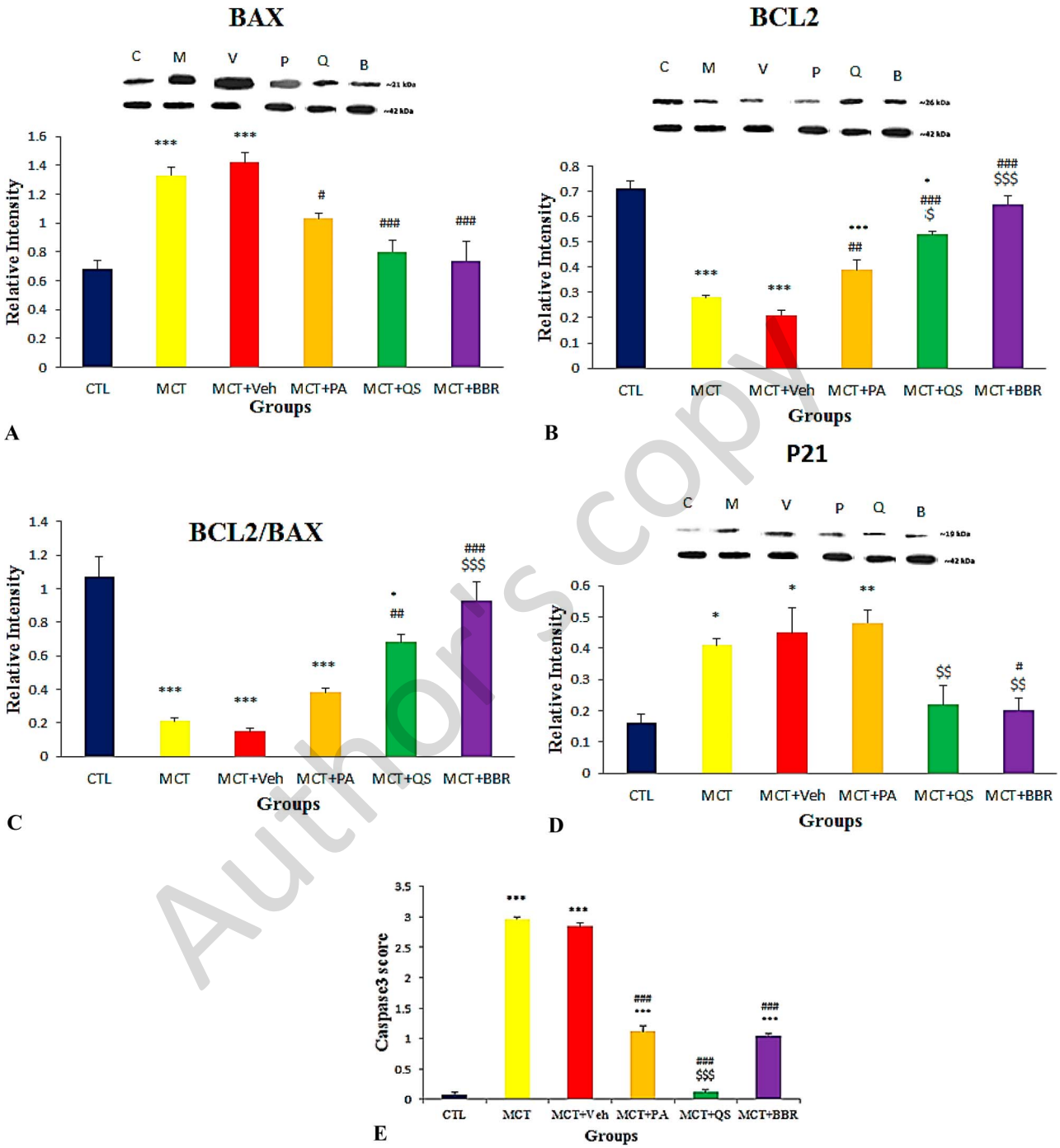

FIGURE 6. Effect of PAH on the expression (mean ± SEM) of Bax (A), Bcl-2 (B), Bcl-2/Bax ratio (C), cell cycle inhibitor p21 (D), and caspase-3 (E) in the RV of the studied groups. The internal control for the expressions of factors in A to D was β-actin. The expression of caspase-3 was assessed by immunohistochemistry staining. ***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$ versus the control group; ###$P < 0.001$, ##$P < 0.01$, and #$P < 0.05$ versus the MCT + Veh group; and \$$P < 0.05$, \$\$$P < 0.01$, and \$\$\$$P < 0.001$ versus the MCT + PA group. n = 6 in each group.

DISCUSSION

In this study, we investigated the effect of 3 plant derivatives, perillyl alcohol (PA), quercetin (QS), and berberine (BBR), on the right ventricle (RV) function, expression of miR-204 and miR-27a, and biochemical factors in RV of experimental PAH in rats. It was shown that all 3 derivatives can effectively protect the RV against hypertrophy, fibrosis, inflammation, apoptosis, and oxidative stress in PAH.

Luan et al[28] reported that baicalin, a natural flavonoid, can have protective effects against heart and lung damage in

PAH rats through the reduction of the level of inflammatory factors IL-6 and TNFα in the RV. Although the role of inflammation is very prominent in vascular remodeling of the lung, the effect of inflammation is also significant in remodeling and failure of the RV. Some studies have shown evidence of RV inflammation in PAH.[2,4] In line with these findings, we observed that the levels of the 2 important proinflammatory cytokines TNFα and IL-6 were significantly increased in the RV of PAH rats. TNFα is involved in contractile dysfunction by impairing the β-adrenergic receptor coupling and by reactive oxygen species formation, inducible nitric oxide synthase expression, and reducing contractile proteins in cardiomyocytes.[29] IL-6 is also involved in cardiac cell contractile dysfunction by reducing sarcoplasmic calcium ATPase (SERCA) pumps in isolated cardiomyocytes and reducing the expression of alpha-myosin and beta-myosin heavy chains.[5,30]

TNFα can induce myocardial hypertrophy[6,31] and initiate apoptosis in cardiomyocytes by activating matrix metalloproteinase (MMP) and reducing the tissue inhibitor of MMP in vivo and in vitro.[29,32] Based on the data obtained from this study, apoptotic proteins Bax and caspase-3 increased in the RV and the antiapoptotic protein Bcl-2 decreased. Several studies have shown that unlike the lung in which apoptosis decreases during PAH, this phenomenon increases in the impaired RV and cell growth stops.[29,30] In line with these findings, we observed that the cell cycle inhibitor protein p21 increased in the right ventricle of the MCT-induced PAH rats.

In this study, PA, QS, and BBR were able to reduce ventricular fibrosis and eliminate hypertrophy. Moreover, after treatment with these plant derivatives, antiapoptotic protein Bcl-2 increased, the increased Bcl-2/Bax ratio was restored to its normal level, and caspase-3 expression decreased. Considering the increased inflammation in the RV of the PAH rats, TNFα and IL-6 seem to be involved in the development and progression of RV failure in the form of ventricular fibrosis, hypertrophy, and decreased contractile strength. These cytokines may have also induced apoptosis in the right ventricle. PA, QS, and BBR could significantly decrease the expression of TNFα and IL-6, RV fibrosis, hypertrophy, and apoptosis and ameliorate the cardiac complications of the disease.

miR-204 expression decreased in the hearts of the PAH animals, and treatment with PA, QS, and BBR for 3 weeks restored it to the control level. A previous study revealed that miR-204 is an antiapoptotic miRNA.[33] Accordingly, in this study, we observed that the reduction of miR-204 expression in the right ventricle was accompanied by an increase in apoptotic markers. In addition, after treatment with PA, QS, and BBR, miR-204 expression restored and apoptosis was decreased. Therefore, these plant derivatives can reduce cardiac apoptosis, probably by restoration of miR-204 expression. In our previous study in the lung,[20] similar to this study in the heart, miR-204 decreased in the PAH rats. However, apoptosis decreased in the lung, whereas it increased in the heart. It is not known whether miR-204 uses the same pathway to cause apoptosis in the lung and heart. Yung et al[33] reported that a microRNA that is antiapoptotic in one cell type could be apoptotic in another cell type.

In this study miR-27a expression in the right ventricle of PAH rats did not change significantly. However, Zhuang et al[13] showed that miR-27a mediates the myocardial fibrosis in rats with chronic heart failure through the Wnt/β-catenin pathway. It seems that either the effect is depending on the type of cardiac failure or the time course of inducing failure. Here, although the change of the level of this microRNA was

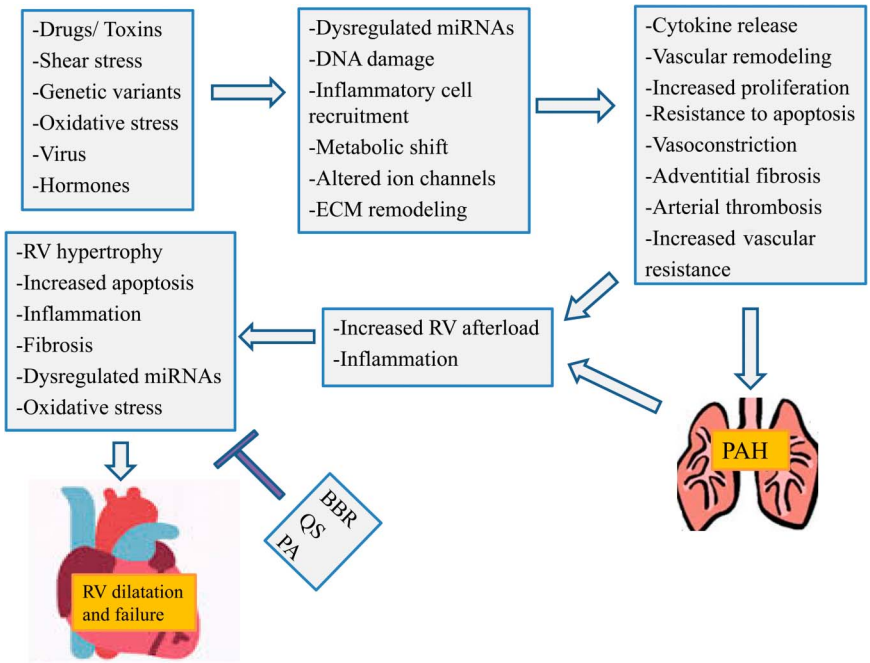

FIGURE 7. Different causes and the mechanisms of progression of pulmonary arterial hypertension (PAH) and its deleterious effects on the lung and heart. The mechanisms by which berberine (BBR), quercetin (QS), and perillyl alcohol (PA) induce their ameliorative affects are also proposed. ECM, extracellular matrix; RV, right ventricle.

not significant, it showed an increasing trend (Fig. 3). Perhaps, the duration of the study was not long enough or miR-27a expression occurs more slowly in this model of cardiac failure. In a study, it was reported that at the beginning of isoproterenol-induced and aldosterone-induced hypertrophy, miR-23a acts first and then miR-27a level increases.[34]

In this study, the level of MDA oxidant in MCT-PAH rats increased while the TAC decreased leading to the increased MDA-to-TAC ratio. Treatment with PA, QS, and BBR for 3 weeks restored this ratio and increased the antioxidant capacity while the level of antioxidants catalase and glutathione peroxidase did not change significantly. Therefore, the increase in TAC by the 3 herbal derivatives may infer that other antioxidants (nonenzymatic ones), that we did not measure, have probably increased.

Overall, we may conclude that QS, PA, and BBR have induced their improving effects by increase in TAC and altering the balance between antioxidant/oxidant levels in the favor of antioxidants, in addition to their anti-inflammatory, antifibrotic, and antiproliferative effects (Fig. 7). The comparison between the 3 herbal derivatives shows that each of them is more effective on a different marker. For instance, BBR was more effective in reducing fibrosis and p21 and BBR and QS were more effective in ameliorating cardiac apoptosis than PA.

CONCLUSION

The results showed that reduction in miR-204 is a probable initiating factor in cardiac complications of PAH. QS, PA, and BBR enforce their anti-inflammatory, antiapoptotic, and antifibrotic effects by normalizing the level of miR-204. They also improve ventricular complications induced by PAH by improving the antioxidant/oxidant balance. Considering that these plant derivatives are present in our daily diet, and QS have already been introduced to the market as a medicinal supplement, they may be introduced as therapeutic goals in the treatment of this debilitating cardiopulmonary disease.

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