A J M S

Online ISSN (2789-3219)

Research Article

Effect of Nicorandil on Endothelial Markers and Tissue Remodeling in Pulmonary Arterial Hypertension Model of Male Rats

Taha Hashim Ahmed[1]* iD , Bahir Abdul-Razzaq Mshimesh[1] iD , Israa Burhan Raoof [2] iD

[1]Department of Pharmacology and Toxicology, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq;
[2]Department of Clinical Laboratory Sciences, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq

Abstract

Background: Chronic pulmonary arterial hypertension (PAH) is a rare, long-lasting illness that makes pulmonary artery endothelial cells (PAEC) not work properly and leads to heart failure and death. *Objective*: To evaluate the effect of nicorandil in the treatment of PAH compared to tadalafil in a rat model of monocrotaline-induced PAH. *Methods*: Monocrotaline injection (60 mg/kg) was used for the induction of PAH in male rats; healthy control and induction groups were not treated. The other 4 groups were treated with either nicorandil or tadalafil with or without treatment blockers (glimepiride and N-Nitroarginine methyl ester (L-NAME)) for 21 days orally. Serum was obtained for assessment of endothelin-1 (ET-1) and tissue harvested for nuclear factor kappa B (NFκB) by ELISA, western blot analysis of endothelial nitric oxide synthase (eNOS), and an apoptosis assay to examine the endothelial function. *Results*: Nicorandil showed a significant reduction in ET-1 and significant elevation in eNOS compared to the induction group, with comparable efficacy to tadalafil; blocker groups showed significantly elevated levels of ET-1 and reduced levels of eNOS compared to healthy control; NFκB was significantly inhibited in nicorandil and tadalafil groups and significantly elevated in blocker and induction groups; while in the TUNEL apoptosis assay, nicorandil showed the highest level of inhibition to apoptosis with apparently normal endothelium lining. *Conclusions*: Nicorandil shows anti-inflammatory, antiapoptotic, and enhanced endothelial morphology and function compared to the induction model.

Keywords: Anti-apoptotic effect, Endothelin-1, Nicorandil, NFκB, Nitric oxide, Pulmonary arterial hypertension.

<div dir="rtl">

تأثير نيكورانديل على الواسمات البطانية وإعادة تشكيل الأنسجة في نموذج ارتفاع ضغط الدم الشرياني الرئوي لذكور الجرذان

الخلاصة

الخلفية: ارتفاع ضغط الدم الشرياني الرئوي المزمن (PAH) هو مرض نادر وطويل الأمد يجعل الخلايا البطانية للشريان الرئوي (PAEC) لا تعمل بشكل صحيح ويؤدي إلى فشل القلب والوفاة. **الهدف**: تقييم تأثير نيكورانديل في علاج PAH مقارنة ب tadalafil في نموذج الجرذان من PAH الناجم عن مونوكروتالين. **الطرق**: تم استخدام حقن أحادي الكروتالين (60 مغ / كغ) لتحريض PAH في ذكور الجرذان. لم يتم علاج مجموعات التحكم والحث الصحية. عولجت المجموعات ال 4 الأخرى إما بنيكورانديل أو تادالافيل مع أو بدون حاصرات علاجية مثل غليميبيريد وإستر ميثيل نيتروارجينين لمدة 21 يوما عن طريق الفم. تم الحصول على المصل لتقييم الإندوثيلين -1 والأنسجة التي تم حصادها للعامل النووي كابا ب (NFκB) بواسطة ELISA ، وتحليل اللطخة الغربية لمصنع أكسيد النيتريك البطاني (eNOS) ، ومقايسة موت الخلايا المبرمج لفحص وظيفة البطانة. **النتائج**: أظهر نيكورانديل انخفاضا كبيرا في ET-1 وارتفاعا كبيرا في eNOS مقارنة بمجموعة الحث، مع فعالية مماثلة للتادالافيل. أظهرت مجموعات الحاصرات مستويات مرتفعة بشكل ملحوظ من ET-1 ومستويات منخفضة من eNOS مقارنة بالتحكم الصحي ؛ تم تثبيط NFκB بشكل كبير في مجموعتينيكورانديل و تادالافيل وارتفاع كبير في مجموعات الحاصرات والتحريض. أثناء اختبار موت الخلايا المبرمج TUNEL ، أظهر نيكورانديل أعلى مستوى من تثبيط موت الخلايا المبرمج مع بطانة بطانة طبيعية على ما يبدو. **الاستنتاجات**: أظهر نيكورانديل تحسنا في مورفولوجيا ووظيفة بطانة الشرايين وفعالية مضادة للالتهابات ومحسنة لوظائفها مقارنة بنموذج الحث.

</div>

* *Corresponding author*: Taha H. Ahmed, Department of Pharmacology and Toxicology, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq; Email: ta.ahmed37@gmail.com

INTRODUCTION

Pulmonary arterial hypertension (PAH) is group 1 of pulmonary hypertension (PH), according to the World Health Organization (WHO). It was first described by the German physician Ernst von Romberg in 1891, and the complete pathophysiology is still unknown [1]. It is a progressive, chronic, and deadly disease of the pulmonary arteries characterized by extensive pulmonary artery constriction accompanied by vascular endothelial and vascular smooth muscle changes and an increase in mean pulmonary arterial pressure above 25mm Hg; however, PAH is considered a rare disease [2,3]; The prevalence and incidence of PAH according to the Registry to Evaluate Early and Long-term PAH (REVEAL) in the US, which uses catheterization as the standard of diagnosis, were 15% and 2.4 per year, respectively [4]. In this registry, the 5-year survival rate was high compared with a previous study [5]. In an already-diagnosed patient, it was above 90%, 76%, and 65% for 1,3, and 5 years, respectively, while with recently-diagnosed patients, it was 86% for 1 year, 68% for 3 years, and 61% for 5 years [6]. These advancing pulmonary vascular disorders are triggered by the disruption of the three critical signaling pathways, nitric oxide (NO), prostacyclin (PGI2), and thromboxane A2 (TXA2), as well as the increase of endothelial and smooth muscle endothelin A receptors and reduced endothelin B receptors, together with the elevation of endothelin-1 (ET-1). Endothelial nitric oxide synthase (eNOS) dysfunction, reduced PGI2 production (cyclooxygenase-2 imbalance), and the vasoconstrictive and mitogenic consequences of a raised endothelin-1 signaling pathway all contribute to PAH [7,8]. Nicorandil is a drug used for angina pectoris. It acts by two mechanisms: firstly, as a NO donor, and secondly, as a K^+-ATP channel opener, resulting in vasodilation due to smooth muscle relaxation [9,10]. Although potassium channels play an important role in PAH development, there is no current treatment for these channelopathies. Monocrotaline is a pyrrolizidine alkaloid derived from the Crotalaria spectabilis plant [11] and was historically utilized for inducing PAH in rats with only a single subcutaneous injection. N-nitroarginine methyl ester (L-NAME) is used as an endothelial nitric oxide synthase inhibitor and glimepiride as an ATP-dependent potassium channel blocker. The point of this study is to find out how well nicorandil works as a medicine to treat PAH in rats that have been given monocrotaline.

METHODS

Study design

In this experimental animal study, sixty Wistar albino male rats were used, obtained from the Animal House of the Iraqi Center for Cancer and Medical Genetic Research (ICCMGR) at Mustansiriyah University. They were handled according to the Ethics Committee in the College of Pharmacy, Al-Mustansiriyah University. Rats weighing 200–250 g were maintained in an animal house at 22°C under a 12-hour dark-light cycle and were allocated into 6 groups based on age, and weight-matched animals were selected. Sixty Wistar albino male rats were distributed in 6 groups, 10 in each, as follows: (I) negative control group injected 0.5 ml/kg normal saline subcutaneously (S.C) single dose, then 5% carboxyl methyl cellulose (CMC) orally for 21 days, (II) induction group induced pulmonary arteries remodeling by S.C injection of monocrotaline (MCT) (60 mg/kg/day) at day1 as a single dose, then 5% CMC solution orally for 21 days, (III) nicorandil group induced pulmonary arteries remodeling by S.C injection monocrotaline (MCT) (60 mg/kg/day) at day1 as a single dose, then nicorandil (10 mg/kg) once daily by oral gavages for 21 days, (IV) standard group induced pulmonary arteries remodeling by S.C injection monocrotaline (MCT) (60 mg/kg/day) at day1 as single dose, then tadalafil (10 mg/kg) once daily by oral gavages for 21 days, (V) nicorandil with blocker group induced pulmonary arteries remodeling by S.C injection monocrotaline MCT (60 mg/kg/day) at day1 as a single dose, then nicorandil (10 mg/kg) once daily by oral gavages for 21 days, pretreated with NOS inhibitor (L-NAME) 1mg/ml in drinking water, and ATP-dependent K^+-channel blocker (glimepiride) at a dose of (5mg/kg/day) orally 1 hour before nicorandil, (VI) tadalafil with blocker group induced pulmonary arteries remodeling by S.C injection monocrotaline (MCT) (60 mg/kg/day) at day1 as a single dose, then tadalafil (10 mg/kg) once daily by oral gavages for 21 days, pretreated with NOS inhibitor (L-NAME) 1mg/ml in drinking water, and ATP-dependent K^+-channel blocker (glimepiride) at a dose of 5mg/kg/day orally, 1 hour before tadalafil.

Preparation and drug doses

Monocrotaline was prepared by dissolving monocrotaline in 3 ml of 1 N HCl and then 10 N NaOH to prepare a solution of 7.4 pH. The volume was then completed by distilled water to prepare a solution of monocrotaline containing 20 mg/ml. [12] Groups II to VI were injected S.C. in the ventral thorax. Nicorandil was prepared by dissolving nicorandil in a 5% solution of CMC to prepare a final concentration containing 10 mg/mL of nicorandil. It was given by oral gavage at a dose of 10 mg/kg/day [13] for 21 days to groups III and V. Tadalafil solution was prepared by dissolving 10 mg in 1 ml of 5% CMC solution. It was given orally at a dose of 10 mg/kg/day [14] to groups IV and VI. N-Nitroarginine methyl ester (L-NAME) is fed to animals in drinking water at a dose of 1 mg/ml to groups V and VI [15]. Glimepiride solution was prepared by dissolving 10mg in 1 ml of 5% CMC solution. It was given orally at a dose of 5 mg/kg/day to groups V and VI [16].

Sample collection

For serum collection, 5 ml of blood was taken from each animal by heart acupuncture in each of the six groups 21 days after the induction to measure ET-1 levels. The blood was then placed in a clot-activating tube and centrifuged at 3000x for 15 minutes to prepare the serum. For ELISA analysis, the serum was kept at a temperature of -80 °C. For lung harvest, rats were sacrificed under ketamine/xylazine (at a dose of 80/8 mg/kg) anesthesia,

and the lungs were immediately removed and thoroughly cleaned with distilled water. Then the lungs were divided into 2 parts, one buffered in neutral formalin at 10% for the TUNEL apoptosis assay, and the second part incubated immediately in a deep freeze (-80) for western blot analysis (eNOS) and ELISA (NFκB).

Enzyme-linked immunosorbent assay

After the preparation of serum and tissue samples, they were kept at -80 °C until the time of analysis. The biotinylated antibodies of endothelin-1 (ET-1 ELISA kit MBS263783) and nuclear factor kappa B (NFκB ELISA kit MBS453975) were purchased from My BioSource, USA, and the procedure was followed by the manufacturer's guidelines [17].

Western blot analysis

The Western blot technique was used to measure the concentration of eNOS in lung tissues. Samples were prepared for SDS-PAGE, the protein samples were diluted in a 5x dodecyl sulfate polyacrylamide (SDS) solution and heated at 90 °C for 10 minutes. The heated samples were centrifuged at 4 °C for 2 min at 12000 rpm, and the upper layer was equally loaded into the wheels of 8% gel together with the pre-stained protein [19]. (endothelial nitric oxide synthase (eNOS) monoclonal antibody E-AB-22088) and BCA kit E-BC-K318-M) purchased from Elabscience, the procedure was followed according to the manufacturer's guidelines. By using ImageJ software, the band intensities (concentration of proteins) were measured, and by multiplying the intensity of the targeted protein over the housekeeping protein producing relative intensity to each group, this relative intensity was then multiplied by the relative intensity of the control to calculate the number of folds in comparison to the control (Figure 1). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) polyclonal antibody was used as a standard housekeeping protein [18].
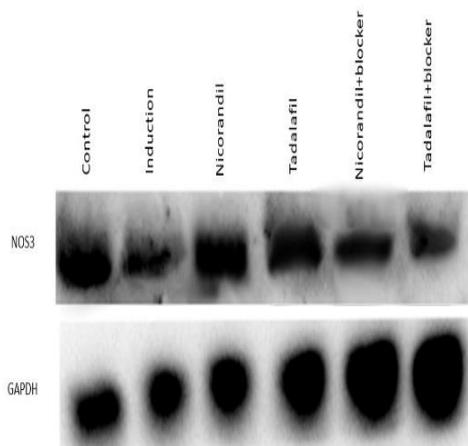
Figure 1: Endothelial nitric oxide synthase (NOS-3) and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression on PVDF membrane of the studied groups.

One-step TUNEL apoptosis assay

Following the fixation, samples were labeled and placed in blocks of paraffin wax according to manufacturer protocol (one-step TUNEL in situ Apoptosis Kit (Green, FITC) fluorescence E-CK-A320, Elabscience, USA). Apoptotic cells were detected utilizing a fluorescent microscope with a FITC filter from Zeiss-Germany and counted computationally by ImageJ macro software; the green fluorescent color indicates the presence of apoptotic fragments [19].

Statistical analysis

Statistical analysis was done utilizing SPSS-20® and Microsoft Excel 2022. Mean and standard deviations were calculated. Analysis of variance (ANOVA) and *post hoc* Tukey test were used for comparing differences among groups. Statistical significance was considered at $p < 0.05$.

RESULTS

The levels of endothelin-1 were significantly lower ($p < 0.05$) in the nicorandil and tadalafil groups (50.06 ± 5.88 and 42.1 ± 1.21 ng/ml, respectively) compared to the induction group (199.08 ± 11.49). The nicorandil blocker and tadalafil blocker groups were about the same in terms of ET-1 levels reduction compared to nicorandil or tadalafil alone ($p < 0.05$), as shown in (Figure 2). The number of folds for each sample showed how the treatment changed the levels of endothelial nitric oxide synthase (eNOS) in lung tissue.
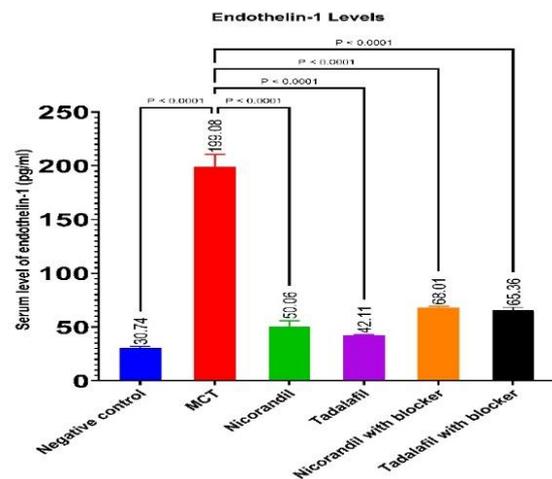
Figure 2: Serum levels of Endothelin-1 among studied groups compared to the induction group. Data are presented as means±SD. Statistically significant at $p < 0.05$ (ANOVA followed Tukey test).

Figure 3 shows that the level of eNOS expression was greatly decreased by 59% in the induction group (0.41-fold) compared to the control (1-fold), while treatment with 10 mg/kg/day of nicorandil elevated eNOS to 84% of the control group. Tadalafil at a dose of 10mg/kg/day only showed 61% of that for the control group, nicorandil with blocker group and tadalafil with blocker group

showed lower levels of eNOS expression (24 and 16%), respectively.
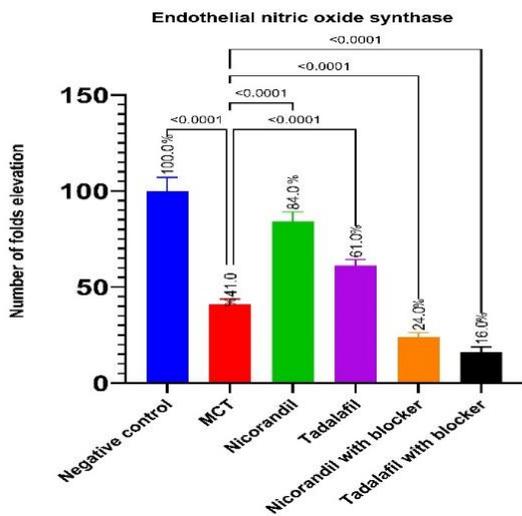
Figure 3: Number of folds expressed as percentages for endothelial nitric oxide synthase (eNOS) expression in studied groups. Statistically significant at *p*<0.05 (ANOVA followed Tukey test).

The effect of treatment on nuclear factor kappa B (NFκB) expression in lung tissue of rats as measured by ELISA showed a significant reduction (*p*<0.05) of NFκB tissue levels (Figure 4) in both nicorandil and tadalafil groups (1.8±0.09 and 1.57±0.13 ng/ml, respectively) in comparison to induction (5.93±0.09 ng/ml). The most reduction was seen in the tadalafil group in comparison to all studied groups. The nicorandil with blocker group and the tadalafil with blocker group had significantly (*p*<0.05) higher levels of NFκB (2.31±0.13 and 2.19±0.12 ng/ml, respectively) compared to the nicorandil or tadalafil alone group and were not statistically different from each other (*p*=0.727).
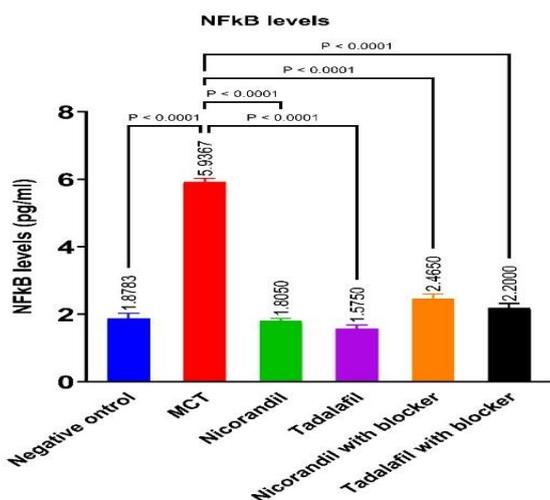

Figure 4: Nuclear Factor Kappa B (NFκB) levels treated groups compared to the control group. Data are mentioned as means±SD. Statistically significant at p<0.05 (ANOVA followed Tukey test).

The effect of treatment on endothelial apoptosis of pulmonary arteries is shown in Figure 5.
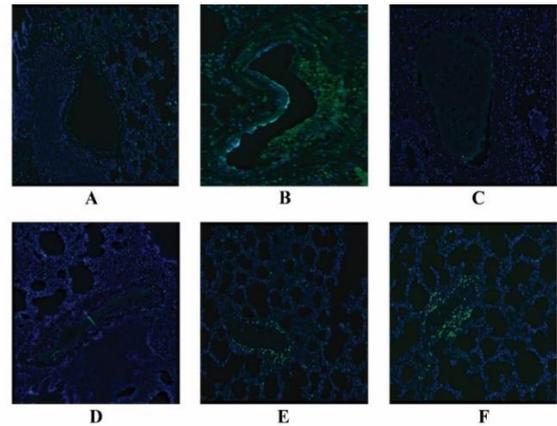
Figure 5: one-step TUNEL apoptosis assay of pulmonary arteries shows apoptotic fragments as green fluorescent. Control group (A), induction group (B), nicorandil group, tadalafil group (D), nicorandil blocker group (E), and tadalafil blocker group (F).

Results illustrated in Figure 6 showed that the apoptotic rate was highest in the pulmonary arteries of the induction group (564.33 cell fragments), while administration of nicorandil or tadalafil suppressed this apoptosis to only 5 and 26 cell fragments, respectively, in pulmonary arteries. The effect of blockers on either nicorandil or tadalafil was obvious in elevating the rate of apoptosis up to 76 and 156, respectively.
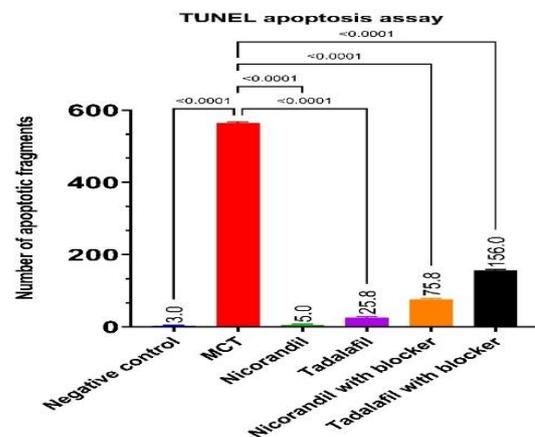
Figure 6: Number of apoptotic fragments in pulmonary arteries for the studied animal groups using TUNEL assay. Data are mentioned as means±SD. Statistically significant at p<0.05 (ANOVA followed Tukey test).

DISCUSSION

Pulmonary arterial hypertension (PAH) is a rare, fast-progressing, incapacitating, and potentially fatal pulmonary vascular illness. Pulmonary vasoconstriction, vascular remodeling, endothelial dysfunction, smooth muscle proliferation, and thrombosis are all components of PAH's pathological mechanisms. These modifications elevate the resistance of the pulmonary arteries, which leads to right heart failure and then death. Potassium channel channelopathies are now recognized as a

pathogenic cause for the development of PAH. Two channelopathies have been recognized due to genetic alterations in genes encoding for potassium channel subfamily K$^+$ member 3 (KCNK3) and ATP-binding cassette subfamily C member 8 (ABCC8). [20] In this study, the administration of monocrotaline (MCT) to induce PAH significantly elevated the level of ET-1. This elevation is consistent with a previous study done by Nagendran *et al*. (2013), who reported an elevation of ET-1 and endothelin-A receptors [21]. This can explain the increased vasoconstriction and endothelial dysfunction in PAH. Nicorandil decreased the level of ET-1, which corresponds to the previous study done by Chen *et al*. (2015), which found that nicorandil decreased the level of ET-1 and elevated the level of NO in patients with coronary artery disease [22]. This reduction of ET-1 can be attributed to ATP-K$^+$ and the known effect of nicorandil, which increases the level of NO [23]. Nitric oxide contributes to the regulation and synthesis of ET-1. The administration of nitric oxide donors down-regulates the synthesis of ET-1 by suppressing the gene responsible for its production [22,24]. The Tadalafil group showed the same results because tadalafil also increases the level of NO in the endothelial tissue due to its PDE5-inhibiting activity. In the blocker groups, the results were intermediate between treatment groups (nicorandil and tadalafil) and the induction group; however, there was a significant difference because L-NAME suppressed the production of nitric oxide, which led to overexpression of ET-1. This went along with a previous study done by Takahashi *et al*. (2005), in which L-NAME increased the level of ET-1 [25]. It has been shown that endothelial NO prevents hyperplasia of vascular smooth muscle cells (VSMCs) and that under hypoxic conditions, the expression of eNOS genes is reduced, suggesting that endothelial dysfunction can be attributed to eNOS reduction [26]. Monocrotaline (MCT) in the induction group of the current study decreased the level of eNOS significantly compared to the control group. In previous studies, it was found that MCT reduced both the expression and activity of eNOS [27,28]. Nicorandil elevated the level of eNOS to approximate that of the control (0.84-fold). Refaie *et al*. (2020) showed that potassium channel openers elevate eNOS through ATP-sensitive potassium channels [29]. Additionally, protein kinase C reduces the activity of eNOS by phosphorylating threonine 495 of eNOS, while nicorandil blocks this phosphorylation by the NO pathway, preserving eNOS activity [30]. Accordingly, nicorandil can increase both the quantity and activity of eNOS [30]. Tadalafil increased eNOS compared to the induction group, and it was 0.61-fold greater than that of the control group. This result is consistent with the previous study done by Vignozzi *et al*. (2016), which showed an increase in eNOS after tadalafil administration [31]. However, the exact mechanism is not clear. In this study, it is suggested that the reduction of inflammation through reduction of NFκB (Figure 4) and improvement in endothelial function after administration of tadalafil could be the reason for the elevation of eNOS levels, because tadalafil only potentiates the effect of NO and does not increase the level of eNOS. Blocker groups also showed reduced levels of eNOS in western blotting; L-NAME, together with nicorandil or tadalafil, reduced the level of eNOS, and this suggests that the nitric oxide pathway could indirectly regulate eNOS expression through decreasing ROS and restoring endothelial function [32]. In the pathogenesis of PAH, NFκB plays an important function by increasing ET-1 and chemokine ligand 5 by elevating these mediators' mRNA [33]. In this study, monocrotaline increased the level of NFκB, which is consistent with a study that found that monocrotaline mediates the initiation of an inflammatory response that activates NF-kB signaling through increasing inflammatory mediators [34]. Nicorandil effectively reduced NFκB to reach that of the control group. In line with the previous study, it was found that nicorandil inhibits toll-like receptors (TLR) that are responsible for NFκB activation and its downstream signaling through nucleotide-binding domain leucine-rich-containing family pyrin domain-containing-3 (NLRP) that lead to activation of caspase 1 and pyroptosis [35]. The Tadalafil group also reduced NFκB, suggesting that NO is the regulatory mediator to control NFκB. Tadalafil increases the level of NO in PAH, and nitric oxide has a negative effect on NFκB, which is in tune with the previous study, which found that NO elevation mediates a suppressor action on the activation of NFκB and synthesis in VSMCs [36]. This also applies to nicorandil. In the blocker groups, there was a significant elevation of NFκB; this can be attributed to eNOS inhibition that led to decreased levels of NO, thus the partial loss of nicorandil and the tadalafil effect [37]. Using the TUNEL technique with FITC-fluorescent dye, the nicorandil group showed apparently normal PAECs compared to the control, with almost the absence of apoptosis fragments, which was even better than the tadalafil group. NFκB is a known factor that controls apoptosis in endothelial cells. Overexpression of NFκB can be mediated by different factors; these include reactive oxygen species (ROS) and hypoxia [38]. Nitric oxide regulates the level of NFκB, exerting negative feedback on the synthesis of NFκB. Upregulation of eNOS by nicorandil and tadalafil resulted in inhibition of NFκB and consequent apoptosis [39]. In the current study, glimepiride did not effectively block ATP-K$^+$ channels in the pulmonary arteries, whether sacrolemmal or mitochondrial. This may be due to the difference between sulfonylurea, in which glibenclamide impairs ischemic conditions but glimepiride does not [41], and only L-NAME acted on eNOS. However, the nicorandil blocker group still demonstrated a better result than the tadalafil blocker group. This is in tune with a previous study that found that the second pathway of nicorandil, that is, mK$^+$-ATP channel opening, resulted in decreased production of ROS and prevented ROS activation of NFκB and apoptosis, as well as ROS-mediated apoptosis independent of NFκB [42].

Conclusion

Nicorandil manifested effective anti-apoptotic activity in the vascular endothelium, was anti-inflammatory, and improved vascular function, as represented by decreased levels of ET-1 and elevated levels of eNOS, which is comparable to the standard treatment tadalafil.

Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

REFERENCES

1. Coons JC, Pogue K, Kolodziej AR, Hirsch GA, George MP. Pulmonary arterial hypertension: a pharmacotherapeutic Update. *Curr Cardiol Rep*. 2019;21. doi:10.1007/s11886-019-1235-4.
2. Arnold ND, Pickworth JA, West LE, Dawson S, Carvalho JA, Casbolt H, et al. A therapeutic antibody targeting osteoprotegerin attenuates severe experimental pulmonary arterial hypertension. *Nat Commun*. 2019;10. doi: 10.1038/s41467-019-13139-9.
3. Corssac GB, Bonetto JP, Campos-Carraro C, Cechinel LR, Zimmer A, Parmeggiani B, et al. Pulmonary arterial hypertension induces the release of circulating extracellular vesicles with oxidative content and alters redox and mitochondrial homeostasis in the brains of rats. *Hyperten Res*. 2021;44. doi: 10.1038/s41440-021-00660-y.
4. Dubroff J, Melendres L, Lin Y, Beene DR, Ketai L. High geographic prevalence of pulmonary artery hypertension: associations with ethnicity, drug use, and altitude. *Pulm Circ*. 2020;10. doi: 10.1177/2045894019894534.
5. Raina A, Humbert M. Risk assessment in pulmonary arterial hypertension. *Eur Respir Rev*. 2016;25. doi: 10.1183/16000617.0077-2016.
6. Harrison F, Dave M, Abby P, David B. Five-year outcomes of patients enrolled in the registry to evaluate early and long-term pulmonary arterial hypertension (PAH) disease management (REVEAL). *Chest*. 2015;148:1043–1954. doi: 10.1378/chest.15-0300.
7. Rajagopal S, Yu YRA. The pathobiology of pulmonary arterial hypertension. *Cardiol Clin*. 2022;40:1-12. doi: 10.1016/j.ccl.2021.08.001.
8. Tettey A, Jiang Y, Li X, Li Y. Therapy for pulmonary arterial hypertension: Glance on nitric oxide pathway. *Front Pharmacol*. 2021;12. doi: 10.3389/fphar.2021.767002.
9. Taira N. Similarity and dissimilarity in the mode and mechanism of action between nicorandil and classical nitrates: An overview. *J Cardiovasc Pharmacol*. 1987;10. doi: 10.1097/00005344-198706108-00002.
10. Zhang M, Zhang H, Liu C, Li X, Ling M, Wang Z, et al. Myocardial protective effects of nicorandil on rats with type 2 diabetic cardiomyopathy. *Med Sci Monit Basic Res*. 2018;24. doi: 10.12659/MSMBR.910974.
11. Yap A, Choo Y. Phytochemical studies of Malaysian Crotalaria mucronata. *Open Conf Proc J*. 2015;4. doi: 10.2174/2210289201304010143.
12. Oh JG, Ishikawa K. Experimental models of cardiovascular diseases: Overview. *Method Mol Biol*. 2018;1816. doi: 10.1007/978-1-4939-8597-5_1.
13. Lenz M, Kaun C, Krychtiuk KA, Haider P, Brekalo M, Maier N, et al. Effects of nicorandil on inflammation, apoptosis and atherosclerotic plaque progression. *Biomedicines*. 2021;9. doi: 10.3390/biomedicines9020120.
14. Leong ZP, Hikasa Y. Effects of masitinib compared with tadalafil for the treatment of monocrotaline-induced pulmonary arterial hypertension in rats. *Vascul Pharmacol*. 2019;122-123. doi: 10.1016/j.vph.2019.106599.
15. Sahara M, Sata M, Morita T, Hirata Y, Nagai R. Nicorandil attenuates monocrotaline-induced vascular endothelial damage and pulmonary arterial hypertension. *PLoS One*. 2012;7. doi: 10.1371/journal.pone.0033367.
16. Dong Y, Chen YT, Yang YX, Shou D, Li CY. Urinary metabolomic profiling in Zucker diabetic fatty rats with type 2 diabetes mellitus treated with glimepiride, metformin, and their combination. *Molecules*. 2016;21. doi: 10.3390/molecules21111446.
17. Al-Shawi N, Al-Rakhat I. Effects of hydrochlorothiazide on tenofovir disoproxil fumarate-induced nephrotoxicity in rats. *Iraqi J Pharm Sci*. 2019;28. doi: 10.31351/vol28iss2pp58-64.
18. Al-Khfajy W, Arif I, Al-Sudani B. Role of fasting mimicking diet in farnesoid x receptor for suppressing epithelial-to-mesenchymal transition, cell cycle progression, and viability of prostate cancer cells. *Iraqi J Pharm Sci*. 2023;32. doi: 10.31351/vol32iss1pp115-124.
19. Maidana DE, Tsoka P, Tian B, Dib B, Matsumoto H, Kataoka K, et al. A novel imagej macro for automated cell death quantitation in the retina. *Invest Ophthalmol Vis Sci*. 2015;56. doi: 10.1167/iovs.15-17599.
20. Redel-Traub G, Sampson KJ, Kass RS, Bohnen MS. Potassium channels as therapeutic targets in pulmonary arterial hypertension. *Biomolecules*. 2022;12:2-16. doi: 10.3390/biom12101341.
21. Nagendran J, Sutendra G, Paterson I, Champion HC, Webster L, Chiu B, et al. Endothelin axis is upregulated in human and rat right ventricular hypertrophy. *Circ Res*. 2013;112. doi: 10.1161/circresaha.111.300448.
22. Chen Z, Chen X, Li S, Huo X, Fu X, Dong X. Nicorandil improves myocardial function by regulating plasma nitric oxide and endothelin-1 in coronary slow flow. *Coron Artery Dis*. 2015;26. doi: 10.1097/MCA.0000000000000179.
23. Zhao JL, Yang YJ, Zhang YH, Pei WD, Sun YH, Zhai M, et al. Intravenous nicorandil preserves endothelial junctions by decreasing endothelin-1 via activation of ATP-sensitive K+ channel. *J Vas Dis*. 2007;36. doi: 10.1024/0301-1526.36.3.175.
24. Bourque SL, Davidge ST, Adams MA. The interaction between endothelin-1 and nitric oxide in the vasculature: New perspectives. *Am J Physiol Regul Integr Comp Physiol*. 2011;300. doi: 10.1152/ajpregu.00397.2010.
25. Takahashi K, Komaru T, Takeda S, Sato K, Kanatsuka H, Shirato K. Nitric oxide inhibition unmasks ischemic myocardium-derived vasoconstrictor signals activating endothelin type a receptor of coronary microvessels. *Am J Physiol Heart Circ Physiol*. 2005;289. doi: 10.1152/ajpheart.00667.2004.
26. Janaszak-jasiecka A, Siekierzycka A, Płoska A, Dobrucki IT, Kalinowski L. Endothelial dysfunction driven by hypoxia—the influence of oxygen deficiency on no bioavailability. *Biomolecules*. 2021;11. doi: 10.3390/biom11070982.
27. Varshney R, Ali Q, Wu C, Sun Z. Monocrotaline-induced pulmonary hypertension involves downregulation of antiaging protein klotho and eNOS activity. *Hypertension*. 2016;68. doi: 10.1161/hypertensionaha.116.08184.
28. Lee MY, Tsai KB, Hsu JH, Shin SJ, Wu JR, Yeh JL. Liraglutide prevents and reverses monocrotaline-induced pulmonary arterial hypertension by suppressing ET-1 and enhancing eNOS/sGC/PKG pathways. *Sci Rep*. 2016;6. doi: 10.1038/srep31788.
29. Refaie MMM, Shehata S, El-Hussieny M, Abdelraheem WM, Bayoumi AMA. Role of ATP-sensitive potassium channel (KATP) and eNOS in mediating the protective effect of nicorandil in cyclophosphamide-induced cardiotoxicity. *Cardiovasc Toxicol*. 2020;20. doi: 10.1007/s12012-019-09535-8.
30. Ghosh S, Gupta M, Xu W, Mavrakis DA, Janocha AJ, Comhair SAA, et al. Phosphorylation inactivation of endothelial nitric oxide synthesis in pulmonary arterial hypertension. *Am J Physiol Lung Cell Mol Physiol*. 2016;310. doi: 10.1152/ajplung.00092.2016.
31. Vignozzi L, Filippi S, Comeglio P, Cellai I, Morelli A, Sarchielli E, et al. P-01-067 effects of chronic treatment with tadalafil in monocrotaline-induced pulmonary hypertensive rat model. *J Sex Med*. 2016;13. doi: 10.1016/j.jsxm.2016.03.218.
32. Serizawa K, Yogo K, Aizawa K, Tashiro Y, Ishizuka N. Nicorandil prevents endothelial dysfunction due to antioxidative effects via normalisation of NADPH oxidase and nitric oxide synthase in streptozotocin diabetic rats. *Cardiovasc Diabetol*. 2011;10:105. doi: 10.1186/1475-2840-10-105.
33. Price LC, Caramori G, Perros F, Meng C, Gambaryan N, Dorfmuller P, et al. Nuclear factor κ-B is activated in the pulmonary vessels of patients with end-stage idiopathic pulmonary arterial hypertension. *PLoS One*. 2013;8. doi: 10.1371/journal.pone.0075415.

34. Zhai C, Shi W, Feng W, Zhu Y, Wang J, Li S, et al. Activation of AMPK prevents monocrotaline-induced pulmonary arterial hypertension by suppression of NF-κB-mediated autophagy activation. *Life Sci*. 2018;208. doi: 10.1016/j.lfs.2018.07.018.

35. Chen F, Chen ZQ, Zhong GL, Zhu JJ. Nicorandil inhibits TLR4/MyD88/NF-κB/NLRP3 signaling pathway to reduce pyroptosis in rats with myocardial infarction. *Exp Biol Med*. 2021;246. doi: 10.1177/15353702211013444.

36. Yoshiyuki H, Kikuo K, Steven G. Retraction of: NO suppresses while peroxynitrite sustains NF-κB: a paradigm to rationalize cytoprotective and cytotoxic actions attributed to NO. *Cardiovasc Res*. 2011;92. doi: 10.1093/cvr/cvr223.

37. Caviedes A, Maturana B, Corvalán K, Engler A, Gordillo F, Varas-Godoy M, et al. eNOS-dependent S-nitrosylation of the NF-κB subunit p65 has neuroprotective effects. *Cell Death Dis*. 2021;12. doi: 10.1038/s41419-020-03338-4.

38. Millar MW, Fazal F, Rahman A. Therapeutic targeting of NF-κB in acute lung injury: A double-edged sword. *Cells*. 2022;11. doi: 10.3390/cells11203317.

39. Balligand JL, Feron O, Dessy C. eNOS activation by physical forces: From short-term regulation of contraction to chronic remodeling of cardiovascular tissues. *Physiol Rev*. 2009;89. doi: 10.1152/physrev.00042.2007.

40. Gopalakrishnan M, Molinari EJ, Shieh CC, Monteggia LM, Roch JM, Whiteaker KL, et al. Pharmacology of human sulphonylurea receptor SUR1 and inward rectifier K+ channel Kir6.2 combination expressed in HEK-293 cells. *Br J Pharmacol*. 2000;129:1323-1332. doi: 10.1038/sj.bjp.0703181.

41. Pantalone KM, Kattan MW, Yu C, Wells BJ, Arrigain S, Jain A, et al. The risk of overall mortality in patients with type 2 diabetes receiving glipizide, glyburide, or glimepiride monotherapy: A retrospective analysis. *Diabetes Care*. 2010;33:1224-1229. doi: 10.2337/dc10-0017.

42. Akopova OV. On the impact of K(ATP) channel opening on mitochondrial reactive oxygen species production. *Histol Cytol Embryol*. 2017;1. doi: 10.15761/hce.1000115.