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# Thymoquinone attenuates monocrotaline-induced pulmonary artery hypertension via inhibiting pulmonary arterial remodeling in rats

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ABSTRACT

*Background:* Pulmonary artery remodeling induced by excess proliferation, migration and apoptosis resistance of pulmonary arterial smooth muscle cells (PASMCs) is a key component in pulmonary artery hypertension (PAH). Thymoquinone (TQ) triggers cancer cells apoptosis through multiple mechanisms. In addition, TQ inhibits migration of human nonsmall-cell lung cancer cells and human glioblastoma cells.
*Objectives:* In the current study, we investigated effects of TQ on MCT-induced PAH in rats and its underlying mechanisms.
*Methods:* After 2 weeks of monocrotaline injection (MCT, 60 mg/kg), Male Sprague–Dawley rats received TQ (8 mg/kg, 12 mg/kg, 16 mg/kg) or olive oil per day for 2 weeks. Hemodynamic changes, right ventricular hypertrophy, and lung morphological features were examined 4 weeks later. In addition, TUNEL, PCNA, α-SMA, Bax and Bcl-2 were detected by immunohistochemistry staining. Bax, Bcl-2, cleaved caspase-3, cleaved poly (ADP-ribose) polymerase (PARP) MMP2, MMP9 and activation of p38MAPK and NF-κB were assessed by Western blot.
*Results:* MCT-induced an increase in pulmonary blood pressure and right ventricular hypertrophy, which were attenuated by TQ treatment. TQ also blocked MCT-induced pulmonary arterial remodeling, proliferation of PASMCs, elevation of MMP2 and downregulation of ratio of Bax/Bcl-2, cleaved caspase-3 and cleaved PARP. Furthermore, TQ inhibited MCT-induced activation of p38MAPK and NF-κB.
*Conclusions:* TQ ameliorates MCT-induced pulmonary artery hypertension by inhibiting pulmonary arterial remodeling partially via p38MAPK/NF-κB signaling pathway in rats.


## 1. Introduction

Pulmonary arterial hypertension (PAH) is a complex disorder characterized by extensive obliteration of small and midsized pulmonary arteries leading to elevated pulmonary arterial pressure and right heart failure [1–3]. Currently, prostacyclins, endothelin antagonists, and phosphodiesterase type 5 inhibitors are the primary drugs shown to improve the quality of life of patients and symptoms [4], which focus on dilating the partially occluded vessels and possess weak antiproliferative effects [5]. However, they fail to reverse progress of the disease and prolong life of patients [6]. PAH has a multifactorial pathogenesis, including excessive migration and proliferation of smooth muscle cells (SMCs), dysfunction of endothelial cells (ECs) [7], oxidative stress [5], inflammation [8] and dysregulated immunity [9]. Among them, vascular remodeling induced by PASMCs proliferation and migration plays a vital role in pathobiology of PAH [10]. Therefore, inhibition of PASMCs proliferation and migration will be an efficient therapeutic strategy for PAH.

Thymoquinone (TQ) is the main active constituent of black seed essential oil of the *Nigella sativa* L, which exhibits anti-inflammatory and anticancer effects. TQ was shown to possess analgesic, antidiabetic, and antihistaminic effects, and be able to alleviate respiratory diseases, rheumatoid arthritis, atherosclerosis and hypertension [11–13]. In addition, the anticancer activities of TQ have also been well demonstrated in several animal models and tumor cell lines [14–16]. TQ has been demonstrated to inhibit activation of activator of transcription 3 (STAT3), leading to human multiple myeloma cells apoptosis [17]. TQ's antiproliferative effect has been linked to its capacity to suppress mitogen-activated protein kinase (MAPK) in multiple myeloma [18], squamous cell carcinoma [19], and human prostate cancer cell lines [20]. Furthermore, TQ enhances apoptosis and reduced proliferation of tumors through increasing the Bax/Bcl-2 ratio [21]. TQ has been also shown to induce the deregulation of the Bcl-2 protein family followed by the activation of caspases [22] and consequently the cleavage of the effector of apoptosis, poly(ADP-ribose) polymerase (PARP) in some cancer cells [17]. TQ also inhibits migration of human nonsmall-cell lung cancer cells and human glioblastoma cells and human glioblastoma cells through reducing expression of MMP2, MMP9 [23,24]. Therefore, we hypothesize that TQ may also trigger apoptosis and inhibit migration

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of PASMCs to attenuate pulmonary arterial hypertension in MCT-induced PAH.

In this study, we explored the effects of TQ on PAH and pulmonary arterial remodeling with a rat model of MCT-induced PAH. Moreover, we examined effects of TQ on PAMSCs proliferation and migration in vivo and underlying molecule mechanisms of these effects.

## 2. Methods

### 2.1. Chemicals and reagents

TQ, MCT and bovine serum albumin (BSA) were purchased from sigma (Saint Louis, MO). TQ were stored at 4 °C and dissolved in olive oil. MCT were dissolved in 1 N HCL neutralized with 1 N NaOH and diluted with NS. Antibodies used in the experiments were purchased from the following suppliers: p-p38MAPK, p38MAPK, anti-cleavaged caspase-3, poly(ADP-ribose) polymerase (PARP), peroxidase-conjugated anti-rabbit secondary antibodies from Cell Signaling Technology (Danvers, MA), Bcl-2-like protein 4 (Bax), α-SMA from sigma (Saint Louis, MO), B-cell lymphoma 2 (Bcl2), NF-κB p-p65, inhibitor of κB (IκB), MMP2, MMP9 from Santa Cruz Biotechnology (Santa Cruz, CA), α-smooth muscle actin (SMA) from sigma (Saint Louis, MO). Masson assay kit was purchased from Solarbio (Beijing, China). One-step TUNEL apoptosis assay kit was obtained from Beyotime (Jiangsu, China).

### 2.2. Animal models and experimental design

SD rats weighing 220–250 g were used in present study. Animals were purchased from Experimental animal center of Zhejiang Province. All animal studies decried in this work have been approved by the Wenzhou Medical University Animal Policy and Welfare Committee. All experiments involving rats were carried out according to the National

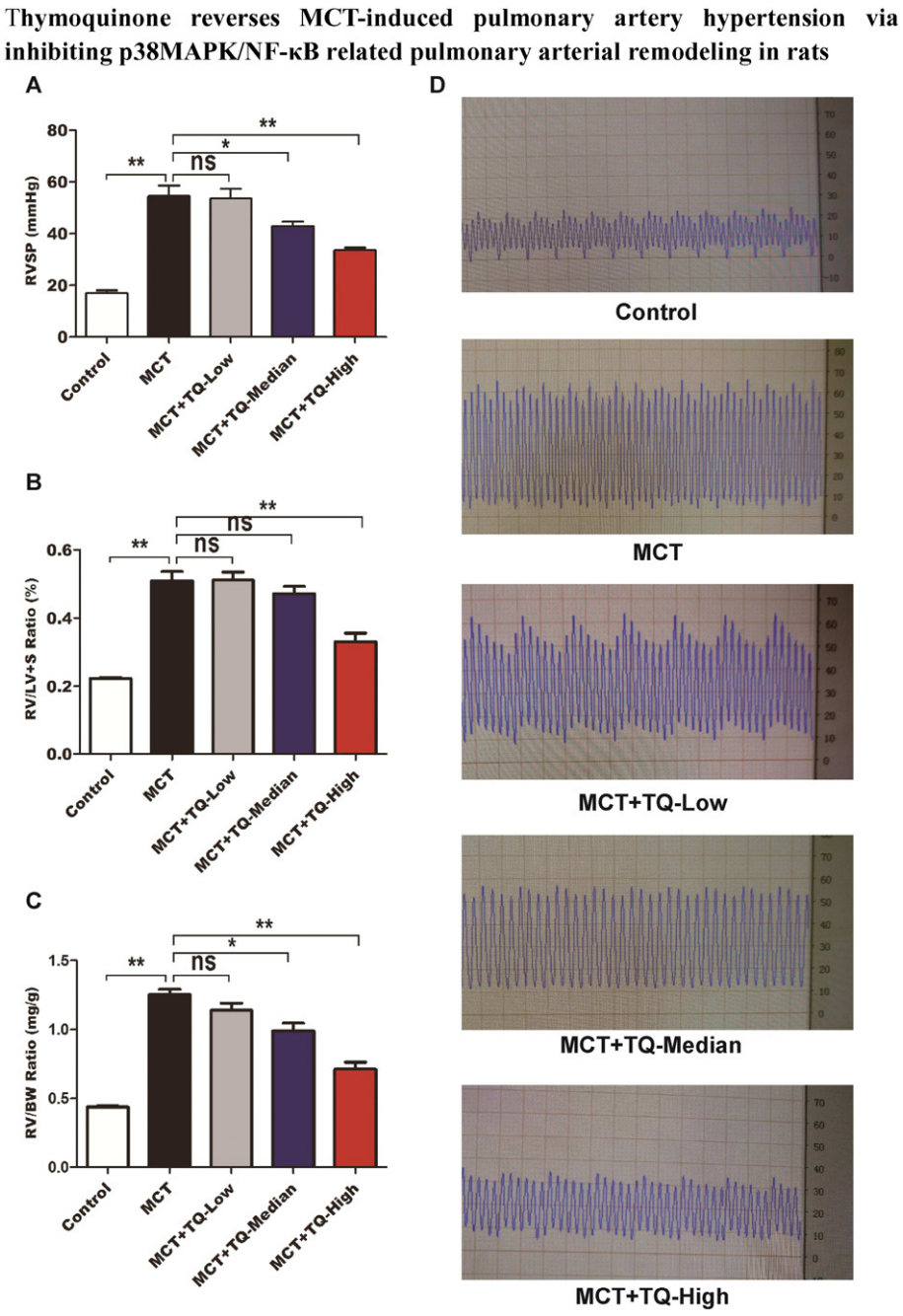


**Fig. 1.** Effects of TQ on hemodynamic changes and right ventricular hypertrophy in MCT-induced PAH. Rats were exposed to saline or MCT with increasing concentrations of TQ (8 mg/kg, 12 mg/kg, and 16 mg/kg). RVSP (A), RV/LV + S ratio (B), and RV / body weight ratio (C) were calculated. Representative pictures of RVSP waves in each groups (D). *$p < 0.05$, **$p < 0.01$; $n = 8$ per group.