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# *p*-Coumaric acid as a prophylactic measure against normobaric hypoxia induced pulmonary edema in mice

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ABSTRACT

*Aims:* Previous studies indicate that the anti-hypoxia effects of Tibetan Turnip (*Brassica rapa* ssp. *rapa*) were closely related to its characteristic components being *p*-coumaric acid (CA) and *p*-coumaric acid-β-D-glucopyranoside (CAG). Since CAG would be converted to CA in vivo, this study aims to further examine the efficacy and mechanism of CA against pulmonary edema induced by normobaric hypoxia.

*Main methods:* Male ICR mice were assigned to the normoxia group and several hypoxia groups, given sterile water, CA or dexamethasone orally, once daily for four consecutive days. One hour after the final gavage, mice in the above hypoxia groups were put into the normobaric hypoxia chamber (9.5% $O_2$) for 24 h while mice in normoxia group remained outside the chamber. After hypoxia exposure, lung water content (LWC), pulmonary vascular permeability, the protein content of bronchoalveolar lavage fluid (BALF), plasma total nitrate/nitrite ($NO_x$) and endothelin-1 (ET-1) content, histological and ultra-microstructure analyses were performed. Expression of occludin was assayed by immunohistochemistry.

*Key findings:* In a hypoxic environment of 9.5% $O_2$, mice treated with 100 mg/kg body wt CA had significantly lower LWC and BALF protein content than mice in the hypoxia vehicle group. Meanwhile, mice in CA group showed intact lung blood-gas-barrier, increased levels of plasma total NO, decreased levels of plasma ET-1 and upregulation of occludin expression.

*Significance:* CA exerts preventive effects against normobaric hypoxic pulmonary edema in mice, its mechanisms involved improving the integrity of the lung barrier, inhibiting oxidative stress and inflammation.

## 1. Introduction

Over 140 million people live at high altitude above 2500 m [1]. Large numbers of low-altitude people travel to high-altitude areas for work or leisure. In addition, troops are deployed to high-altitude areas for patrol, combat training and emergency rescue [2]. Low barometric pressure and related low partial pressure of oxygen (hypoxia), which are features of the high-altitude climate, can induce illnesses including acute mountain sickness (AMS), high altitude pulmonary edema (HAPE) and high altitude cerebral edema (HACE) posing a threat to human health and life [3,4]. High altitude pulmonary edema (HAPE) is more severe than AMS and its incidence is higher than that of HACE. Among individuals with no history of HAPE, the incidence of HAPE is 6% and 15% in the first two days following ascent to altitudes of 4500 m and 5500 m, respectively [5]. Early stage symptoms of HAPE include exertion dyspnea, cough, and reduced exercise performance. As pulmonary edema worsens, symptoms of orthopnea, breathlessness at rest, and gurgling in the chest develop, cough exacerbates, and pink frothy sputum occurs indicating obvious pulmonary edema [6]. Regular measures taken against HAPE include prompt descent to a low-altitude area, oxygen inhalation and administration of medicine such as dexamethasone and nifedipine [7,8]. HAPE can be prevented by acclimatization over several days or prophylactic administration of medicine or functional foods in advance [9].

Turnip (*Brassica rapa* ssp. *rapa*) belongs to *Cruciferae Brassica* and grows widely in the world [10]. Tibetan turnip is a traditional food and medicine used for anti-hypoxia and anti-fatigue by the local Tibetan communities [11]. Previous studies show that turnip is rich in polysaccharides, flavonoids, terpenes, coumarins, and other bioactive compounds, which have demonstrated biological functions such as prevention of oxidative stress, regulation of immune system and lowering risks of cancer [12]. Through isolation, purification and

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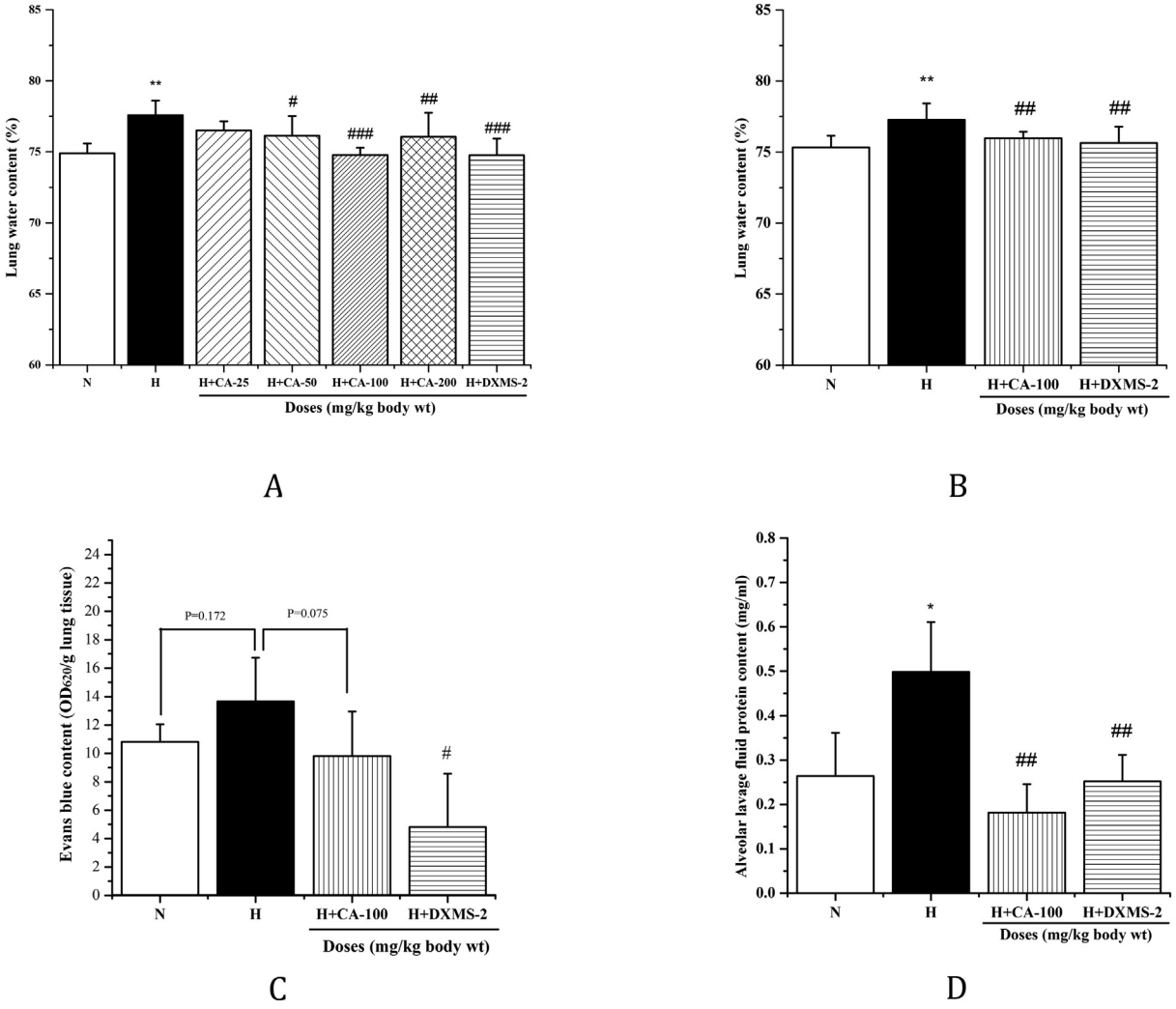


**Fig. 1.** Effect of preventative treatment with CA and dexamethasone on hypoxia induced lung impairment.
(A) LWC of mice treated with different doses of CA and dexamethasone (n = 10); (B) LWC of mice treated with 100 mg/kg body wt CA and dexamethasone (n = 10); (C) effect of CA and dexamethasone on pulmonary vascular permeability (n = 5); (D) effect of CA and dexamethasone on BALF protein level (n = 5). Values were presented as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with normoxia control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared with hypoxia vehicle group. N, normoxia; H, hypoxia; H + CA-25, hypoxia +25 mg CA; H + CA-50, hypoxia +50 mg CA; H + CA-100, hypoxia +100 mg CA; H + CA-200, hypoxia +200 mg CA; H + DXMS-2, hypoxia +2 mg dexamethasone.

identification, our research team found that *p*-coumaric acid (CA) and *p*-coumaric acid-β-D-glucopyranoside (CAG) were the characteristic anti-hypoxia components [13]. Under physiology conditions, CAG would be converted to CA after ingestion. CA is a hydroxy derivative of cinnamic acid widely found in plants and plant-based foods, including tea, bamboo leaves, coffee, wine and beer [14,15]. Previous studies reported that CA possesses immunomodulatory, anti-inflammatory, anti-ulcer, anti-microbial, anti-stomach cancer activities, and can modulate glucose and lipid metabolism, render protection against atherosclerosis and Parkinson's disease [16–18].

Previously, our research group revealed that CA had a potent effect against normobaric hypoxia induced pulmonary edema [13]. In the present study, we aim to explore the capacity of CA to prevent normobaric hypoxia induced pulmonary edema and improve lung barrier function within an acute normobaric hypoxia mouse model. The prevention mechanisms of CA were further discussed.

## 2. Materials and methods

### 2.1. Experimental animals

The experiment was carried out with 150 male ICR mice (Super-B&K Laboratory Animal Corp., Shanghai, China) weighing 25–30 g each. All animal procedures were carried out in accordance with National Institutes of Health guide for the care and use of Laboratory animals and approved by the Committee on the Ethics of Animal Experiments of the Laboratory Animal Research Center, Zhejiang Chinese Medical University (Hangzhou, China) (Authorisation No: ZSLL-2017-060).

### 2.2. Optimization of CA dose

Prophylactic effect and dose-dependent studies of CA against pulmonary edema induced by normobaric hypoxia were carried out in mice. After 3–4 day adaptation, a total of 70 male ICR mice were divided into 7 groups of 10 mice each: (I) normoxia (N), (II) hypoxia (H), (III) hypoxia +25 mg/kg body wt CA (H + CA-25), (IV) hypoxia +50 mg/kg body wt CA (H + CA-50), (V) hypoxia +100 mg/kg body wt CA (H + CA-100), (VI) hypoxia +200 mg/kg body wt CA (H + CA-