Histol Histopathol (2017) 32: 35-41

http://www.hh.um.es

Histology and Histopathology

*From Cell Biology to Tissue Engineering*

# Genistein attenuates monocrotaline-induced pulmonary arterial hypertension in rats by activating PI3K/Akt/eNOS signaling

**Zeqi Zheng[1]*, Songping Yu[1]*, Wan Zhang[1], Yongchao Peng[1], Mingyu Pu[1], Ting Kang[1], Junyi Zeng[2], Yuefei Yu[3] and Guorong Li[4,5]**

[1]The Division of Cardiology, The First Affiliated Hospital, Nanchang University, [2]Jiangxi Institute of Hypertension, Nanchang, China, [3]Ameritech Biomedicines, Inc., Houston, TX, USA, [4]Inserm U1059, Saint-Etienne and [5]CHU Saint-Etienne, Plateau of Biology, France

*These are equal contributors and share the first authorship

**Summary.** Introduction: Phytoestrogen genistein may be useful to treat pulmonary arterial hypertension (PAH). However, its mechanism is still not clear. The aim of the present study was to confirm the therapeutic effects of phytoestrogen genistein on PAH in monocrotaline-induced rat model and to explore its mechanism. Materials and Methods: Sprague-Dawley male rats were randomly divided into 4 groups: control group (n=8), PAH group (n=8), genistein treament group with three different doses (n=8 in each dose group) and group of PI3K inhibitor LY294002. The rat model of PAH was induced by monocrotaline (MCT). The situation of survival of rats was observed. Pathological studies of lung and heart tissues were performed. Western-blot detection of P-Akt and P-eNOS expression levels in lung tissue was carried out. Nitrate reductase analysis was used to measure nitric oxide (NO) in lung tissue. Results: Genistein treatment resulted in significant improvement in the speed of tricuspid regurgitation, diameter of pulmonary artery, mean pulmonary artery pressure and right ventricular hypertrophy index. Genistein treatment also resulted in significant improvement in the stenosis of pulmonary artery, proliferation of smooth muscle, right ventricular hypertrophy and myocardial hypertrophy. These therapeutic effects were more obvious with increasing dose of genistein. After genistein treatment, amelioration in survival rates of PAH rats was observed. PI3K inhibitor LY294002 could block these therapeutic effects. In rat lung tissue, P-Akt, P-eNOS and NO expressions were increased significantly in genistein treatment group when compared with PAH group ($p<0.05$, respectively). The increase in expression level of P-Akt, P-eNOS and NO was correlated with genistein dose. P-Akt, P-eNOS and NO expressions in lung tissue increased slightly in the PI3K inhibitor LY294002 group when compared with PAH group, but the difference was not statistically significant ($p>0.05$). Conclusions: We confirmed that genistein could relax pulmonary vascular resistance, reduce pulmonary artery pressure, improve right heart function and ameliorate survival rate in the rat model of PAH. Our study suggested that its mechanism was related with PI3K/Akt/eNOS signal pathway. Phytoestrogen genistein may become a new and effective drug for patients with PAH.

**Key words:** Pulmonary hypertension, Genistein, Rat model, PI3K/Akt/eNOS signaling pathway, PI3K inhibitor LY294002

*Offprint requests to:* Pr. Zeqi Zheng, The division of Cardiology, The First Affiliated Hospital, Nanchang University, Nanchang 330006, China. e-mail: zeqizheng@126.com

DOI: 10.14670/HH-11-768

## Introduction

Pulmonary arterial hypertension (PAH) is a clinical syndrome with the main feature of a progressive increase in pulmonary vascular resistance. Blood flow through the pulmonary circulation is blocked, leading to pathologically elevated PAH. The consequence is right heart failure and death. Currently, the availability of anti-

PAH drugs is very scarce, restricted to the application of endothelial vasomotor regulatory factors (Galiè et al., 2010). The effectiveness of these drugs is also limited. The long-term prognosis is still poor and 5-year survival rate is still low (Humbert et al., 2010; Agarwal et al., 2012). Moreover, the detailed pathogenesis of PAH is not yet fully elucidated. Common features are the dysfunction of small pulmonary artery, disorder of vasomotor cytokine secretion, and imbalance of pulmonary artery endothelial cells and smooth muscle cells. These processes cause the disorder of proliferation and apoptosis and finally lead to vascular remodeling and clump-like lesions (Rabinovitch, 2008). Nitric oxide (NO) is an important endothelium-derived relaxing factor, which plays an important role in pulmonary vascular regulation. Studies have showed that the pathogenesis of PAH is correlated with the reduction of NO synthesis in tiny pulmonary artery (Zhao et al., 2005).

In recent years, studies have found that estrogen has an effect of protecting pulmonary vessels and of promoting vasodilation. After the binding of ordinary estrogen to estrogen receptor, phosphatidylinositol 3 kinase/Akt (PI3K/Akt) signaling pathway is activated, which regulates the biological activity of the pulmonary vascular system. Phyto-estrogens have an estrogenic effect by binding to estrogen receptors (ER), promoting the synthesis of endothelial nitric oxide synthase (eNOS) and the secretion of nitric oxide (NO), which promote vasodilation (Si et al., 2008). Phytoestrogen genistein (Gen) may also bind to estrogen receptors to exert an estrogen-like effect and its affinity for ERβ is significantly higher than for ERα (Chrzan et al., 2007).

We established a rat model of monocrotaline-induced PAH to verify the effects of phytoestrogen genistein for reducing pulmonary artery pressure and improving right ventricular function. We further explored its mechanism to correlate genistein's effects with PI3K/Akt/eNOS signaling pathway.

## Materials and methods

### *Rat model of PAH*

Sprague-Dawley (SD) rats were provided by Laboratory Animal Center of Nanchang University School of Medicine. The male rats were 3 months old, weighing between 200-250 g. Animal breeding process and experimental methods were in accordance with the " implementing rules on experimental animals" promulgated at national level. 98% monocrotaline (MCT) was used (Sigma, USA). After a single intraperitoneal injection of monocrotaline (50 mg/kg), pulmonary artery pressure, pulmonary artery, tricuspid regurgitation velocity and the change of right ventricular hypertrophy index were determined at day 0, 7, 10 and 21. Pulmonary arterial hypertension was measured at day 21 to continue the next experiment.

### *Treatment of rat PAH*

SD rats were randomly divided into four groups. For the control group (n=8): rats were treated with intraperitoneal injection of saline. For the PAH group (n=8), intraperitoneal injection of MCT (50 mg/kg) was performed. For treatment groups (n=24 rats), rats were randomly divided into three groups (Gen20 group, Gen80 group and Gen 200 group) of different dose treatment with each group of 8 rats. After treatment of single intraperitoneal MCT (50 mg/kg), rats were treated by subcutaneous injection of different genistein dose (Gen20: 20 μg/kg, Gen80: 80 μg/kg and Gen200: 200 μg/kg) at the fourth week for two weeks. For the group of PI3K inhibitor LY294002 (n=8), after the intraperitoneal injection of MCT (50 mg/kg), genistein (80 μg/kg) was given subcutaneously in the morning and the intraperitoneal injection of PI3K inhibitor LY294002 (1 mg/kg) was performed in the afternoon at the fourth week for two weeks.

### *Analysis of therapeutic effects and study of PI3K/Akt/eNOS signaling pathway*

At the end of the fifth week, Doppler echocardiography was used to measure right heart related indicators. Right heart catheterization was used to measure mean pulmonary artery pressure. Weight determination was carried out to measure right ventricular hypertrophy index (RVHI). Hematoxylin and eosin (HE) staining of pulmonary vascular pathology specimens was prepared. P-eNOS antibody and P-Akt antibody (Cell signaling corporation, USA) were obtained for western blot assay to measure related proteins. Nitrate reductase analysis was used to measure NO in lung tissue. The survival rates of rats were analyzed.

### *Statistical analysis*

Survival was calculated by the Kaplan-Meier method, and the resulting curves were compared by using the log-rank test. Non parametrical Mann-Whithney test and $\chi^2$ test were used to analyze the difference between two categorical variables. $p<0.05$ was considered to be statistically significant.

## Results

### *Rat PAH model*

Male SD rats received an intraperitoneal injection of MCT (50 mg/kg). We observed the rat change at the time point of day 0, day 7, day 10 and day 21. Tricuspid regurgitation was detected in the control group at day 21 while tricuspid regurgitation appeared at day 7 in the PAH group (34.6±29.4 cm/s, $p<0.05$). It reached 225.8±12.1 cm/s at day 21 ($p<0.05$). The diameter of

pulmonary artery in rats injected with MCT began to decrease at day 10, from 3.1±0.2 mm in the control group to 2.5±0.3 mm in the PAH group ($p<0.05$), from 2.6±0.2 mm in the control group to 3.3±0.2 mm in the MCT group at day 21($p<0.05$). For the PAH group, mean pressure of pulmonary artery gradually increased from day 10. At day 21, the mean pulmonary artery pressure increased from 12.4±1.2 mmHg in the control group to 27.4±2.8 mmHg in the PAH group ($p<0.05$). It was found that right ventricular hypertrophy indexes in the PAH group were significantly increased when compared with the control group ($p<0.05$) at day 21.These results indicated that rat model of PAH was established at day 21 after the injection of MCT.

*Therapeutic effects of genistein on PAH rats.*

After two weeks' treatment, the tricuspid regurgitation velocity and the diameter of pulmonary artery were measured. It was found that the tricuspid regurgitation velocity was respectively 36.125±14.375 (cm/s), 217.162±47.338 (cm/s), 177.6±52.800 (cm/s), 122.262±38.838 (cm/s), 78.525±45.725 (cm/s), 203.825±46.575 (cm/s) in the control group, PAH group, Gen20 group, Gen80 group, Gen200 group and PI3K inhibitor group. With the increase of genistien dose, the speed of tricuspid regurgitation decreased more significantly ($p<0.05$). For the PI3K inhibitor LY294002 group, the diameter of pulmonary artery and the speed of tricuspid regurgitation were not improved when compared with the PAH group ($p>0.05$).

It was found that in the control group, PAH group, Gen20 group, Gen80 group, Gen200 group and PI3K inhibitor group, the diameter of pulmonary artery was respectively 2.675±0.875 (mm), 3.738±1.938 (mm), 3.425±1.625 (mm), 3.163±1.363 (mm), 2.838±1.562 (mm), 3.675±1.575 (mm). The decrease in the diameter of pulmonary artery was more obvious with the increase in genistein dose ($p<0.05$). For the PI3K inhibitor LY294002 group, when compared with PAH group, the difference in pulmonary artery diameter was not statistically significant ($p>0.05$).

It was found that in the control group, PAH group, Gen20 group, Gen80 group, Gen200 group and PI3K inhibitor group, the mean pulmonary artery pressure was respectively 11.688±7.212 (mmHg), 29.863±11.812 (mmHg), 23.575±9.425 (mmHg), 20.387±8.687 (mmHg), 14.588±6.589 (mmHg) and 27.725±8.725 (mmHg). With the increase of Genistien dose, the mean pulmonary artery pressure was reduced more obviously when compared with PAH group ($p<0.05$). For the PI3K inhibitor group, no significant improvement in the pulmonary artery pressure was found when compared with PAH ($p>0.05$).

*Survival rates of rats*

The survival situation of rats was closely observed. We found that rat death emerged from the 25$^{th}$ day. For the PAH rats, except for one death due to anesthesia, six rat deaths were observed. For the Gen20 group, three rat deaths were observed. For the Gen80 group, two deaths were observed. There was no rat death in the Gen200 group. There was a significant difference in survival rates between each genistein treatment group and PAH group ($p<0.05$). For the PI3K inhibitor group, except for one death due to anesthesia, four deaths were observed. There was no significant difference in survival rate between the PI3K inhibitor group and PAH group ($p>0.05$). The survival rate of rats in each group is demonstrated in Fig. 1.

*Right ventricular hypertrophy index*

It was found that in the control group, PAH group, Gen20 group, Gen80 group, Gen200 group and PI3K inhibitor group, the right ventricular hypertrophy index was respectively 26.525±8.625 (%), 40.238±9.762 (%),

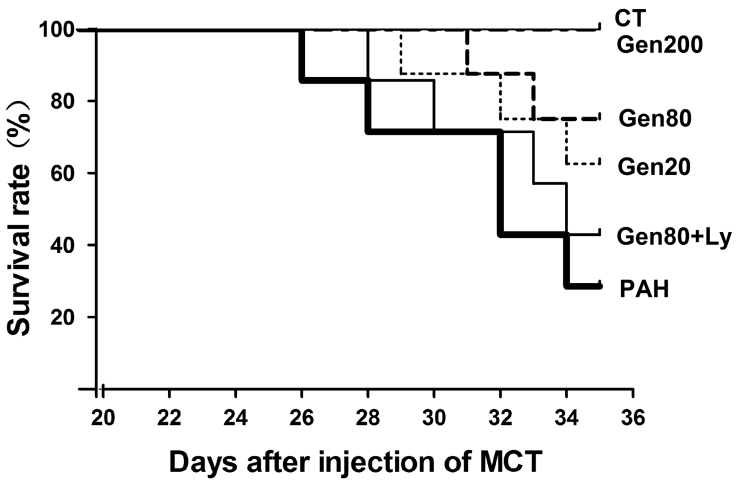


**Fig. 1.** Survival rate of rats in each treatment group. CT for control group; PAH for PAH group; Gen20 for genistein treatment with dose of 20 μg/kg; Gen80 for genistein treatment with dose of 80 μg/kg; Gen200 for genistein treatment with dose of 200 μg/kg and Gen80+Ly for group of PI3K inhibitor LY294002. There was a significant difference in survival rates between each genistein treatment group and PAH group ($p<0.05$).

36.700±9.525 (%), 33.838±7.938 (%), 29.175±9.172 (%), 37.913±9.313 (%). With the increase of genistien dose, the right ventricular hypertrophy index was decreased more significantly when compared with the PAH group ($p<0.05$). For the PI3K inhibitor group, when compared with PAH, the right ventricular hypertrophy index was not improved significantly ($p>0.05$).

### *Pathology observation of pulmonary vascular and right ventricular structure*

After two weeks' genistein treatment, the rats were sacrificed and lung tissue was taken out for HE staining. The stenosis of pulmonary vascular lumen and the proliferation of significant smooth muscle cell were found in the rats of PAH model when compared with the control group (Fig. 2). After two weeks treatment by injection of genistein, pulmonary vascular stenosis was significantly improved in the treatment group when compared with the control group. The improvement was positively correlated with the dose of genistein. There was a significant difference by calculating WT% (percentage of wall thickness and outer diameter) ($p<0.05$) and WA% (tube wall area and the total vessel area) ($p<0.05$). For the PI3K inhibitor group, when compared with the PAH group, the stenosis of pulmonary vascular lumen and the proliferation of significant smooth muscle cell were not obviously different ($p>0.05$).

Rat hearts were removed for HE staining. The same cross-section was selected. The randomly selected sections were compared. It was found that the size of right ventricule and the right ventricular hypertrophy in the PAH group were significantly increased when compared with the control group. For the genistein treatment group, when compared with the PAH group, the right ventricular size and the degree of hypertrophy became smaller. The right ventricular size and the reduction of the degree of hypertrophy were more evident with increasing doses of genistein. For the PI3K inhibitor group, when compared with PAH group, the right ventricular size and the degree of hypertrophy were

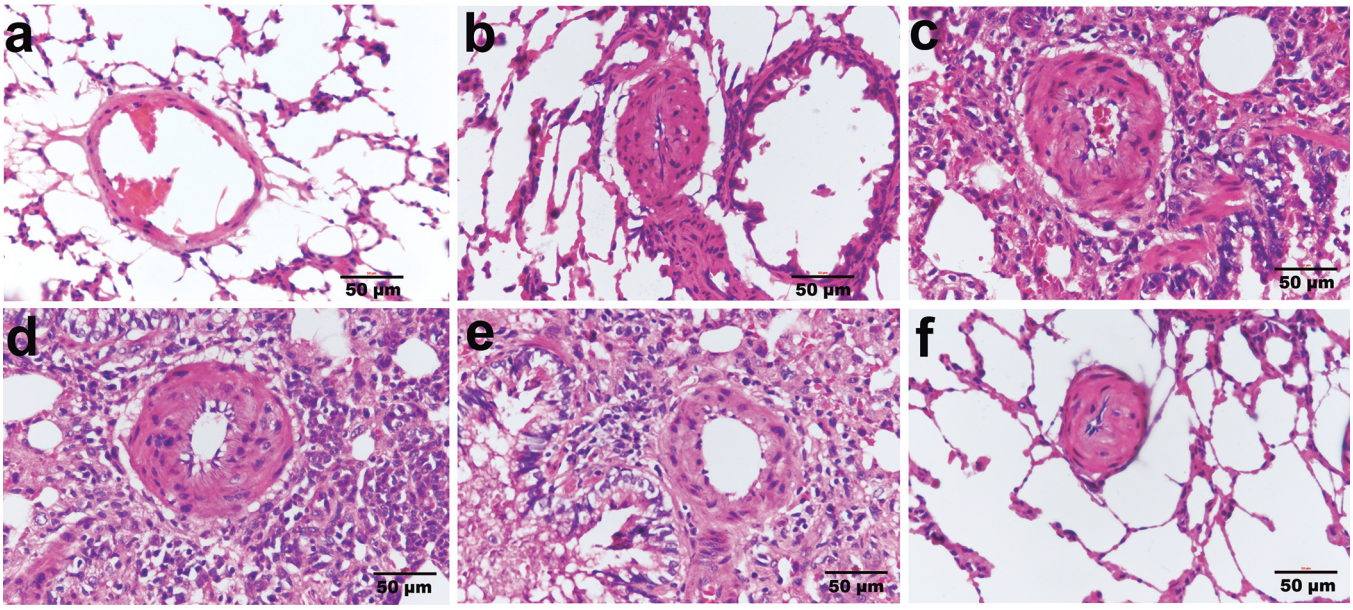


**Fig. 2.** HE staining of lung tissues. HE staining was performed to observe the artery in lung tissue from control group **(a)**, PAH group **(b)**, genistein treatment with dose of 20 μg/kg **(c)**, genistein treatment with dose of 80 μg/kg **(d)**, genistein treatment with dose of 200 μg/kg **(e)**, and treatment with PI3K inhibitor LY294002 **(f)**. Scale bars: 50 μm.

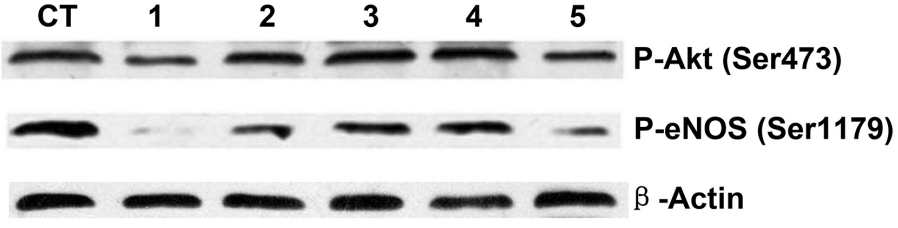


**Fig. 3.** Protein expressions of P-Akt and P-eNOS. Western blot was performed to detect protein expression of P-Akt and P-eNOS in lung tissue. CT for control group; 1 for PAH group; 2 for genistein treatment with dose of 20μg/kg; 3 for genistein treatment with dose of 80μg/kg; 4 for genistein treatment with dose of 200μg/kg and 5 for group of PI3K inhibitor LY294002.

not significantly different.

*Expressions of P-Akt and P-eNOS*

At the end of treatment by genistein, the rats were sacrificed and the lung tissues were removed. The right upper lobe tissue was taken for the extraction of protein. After the measurement of protein concentrations, P-Akt and P-eNOS protein assays were performed. With β-actin as an internal reference, the expression levels of P-Akt and P-eNOS were calculated (Fig. 3). It was found that in the rats of PAH group, when compared with the control group, P-Akt (Ser473) and P-eNOS (Ser1179) expressions were significantly lower ($p<0.05$). In the genistein treatment group, when compared with the PAH group, the expressions of P-Akt (Ser473) and P-eNOS (Ser1179) were significantly higher. A higher expression was observed with the increase of genistein dose ($p<0.05$). For the PI3K inhibitor group, when compared with the PAH group, the expressions of P-Akt (Ser473) and P-eNOS (Ser1179) were slightly elevated, but the difference was not statistically significant ($p>0.05$).

*NO content in lung tissue*

It was found that in the control group, the PAH group, Gen20 group, Gen80 group, Gen200 group and PI3K inhibitor group, the NO amount was respectively 3.637±0.894 (μmol/g), 0.784±0.630 (μmol/g), 1.677± 0.534 (μmol/g), 2.146±0.706 (μmol/g), 3.323±0.907 (μmol/g), 1.250±0.785 (μmol/g). NO amount was significantly lower in the PAH group when compared with the control group ($p<0.05$). With the increase of genistein dose, the increase in NO amount was more obvious ($p<0.05$). For the PI3K inhibitor group, when compared with the PAH group, the NO amount increased slightly, but the difference was not statistically significant ($p>0.05$).

## Discussion

PAH is characterized by the progressive increase of pulmonary vascular remodeling. Recent reports have found that the lower estrogen level is an independent risk factor for the onset of PAH (Lihm et al., 2007; Pugh and Hemnes, 2010). It is well known that estrogen plays a very important role in hormone replacement therapy for cardiovascular disease (Dubey et al., 2005). Phytoestrogen genistein is found to have estrogenic effects. Genistein has gained much attention because of its low prices and fewer side effects.

We used a single injection of MCT (50 mg/kg) to establish rat model of PAH. After the injection of MCT, the azole derivative metabolites of MCT were brought to the pulmonary circulation by the body circulation, causing irreversible injury of pulmonary vascular endothelial cells. Subsequently, rupture in pulmonary vascular elastic membrane, accumulation of extracellular matrix and muscularization of pulmonary artery were induced. Increased resistance in lung vascular, pulmonary vascular remodeling and right ventricular hypertrophy were ultimately produced. Finally, the PAH was formed (Falcetti et al., 2010). Our experiment found that 3 weeks after a single injection of MCT (50 mg/kg) in rats, the diameter of pulmonary artery, tricuspid regurgitation velocity, mean pulmonary artery pressure (mPAP) and right ventricular hypertrophy index (RVHI) were significantly higher than in the control group. We thought that PAH in rats had been formed three weeks after injection of MCT. Our results were in agreement with those reported by Matori et al. (2012). Therefore, our experiment selected the fourth week to begin the treatment for the PAH rats. The treatment consisted of two weeks' continuous subcutaneous genistein. In the mean time, we administered PI3K inhibitor LY294002 to investigate genistein's mechanism.

Our experimental results showed that genistein could improve survival rate among the treated rats. With the increase of genistein dose, more obvious therapeutic effects and higher survival rate were observed. We found that pulmonary artery pressure, right ventricular hypertrophy index, pulmonary artery and tricuspid regurgitation velocity measurement were improved in rats treated with genistein. The degree of improvement was correlated with the increasing doses of genistein. In HE staining of pulmonary vascular and cardiac, we found the improvement of vascular endothelial cell damage, the proliferation of smooth muscle cells, and the increase of the size of the right ventricle and right ventricular hypertrophy in rats of genistein treatment. We confirmed that genistein was effective for the treatment of PAH and that the effect was positively correlated with the dose. We found that genisein therapeutic effects could be blocked by PI3K inhibitor.

Estrogen has been found to improve pulmonary vascular reconstruction and to vasodilate the lung vessels. The protective effect of estrogen on pulmonary vessels is mediated mainly through the estrogen receptor. There are three estrogen receptors: α, β and GPR30 (G protein-coupled estrogen receptor 30). Estrogens combine with the above receptors, activating a number of cellular signaling pathways such as PI3K/Akt pathway, mitogen-activated protein kinase (MAPK) pathway, tyrosine RhoA/Rho kinase pathway, etc (Haynes et al., 2003; Yu et al., 2004; Hiroki et al., 2005; Fitzpatrick et al., 2006). The PI3K/AKT/MAPK signaling pathway plays an important role in the treatment of PAH. Its final step is closely associated with endothelial synthase of nitric oxide (eNOS). Numerous studies have shown that estrogens, by binding to the estrogen receptor, rapidly activate the PI3K/Akt/eNOS signaling pathway (Stirone et al., 2005). This effect may be blocked by estrogen receptor antagonist and by PI3K inhibitor LY294002 (Martin et al., 2008). The phytoestrogen structure is similar to estrogen and has a phenol ring structure. Owing to a phenolic ring structure, phytoestrogens can combine with estrogen receptor α and β. Especially for isoflavones, its affinity with

estrogen receptor β is much higher than with estrogen receptor α (Chrzan et al., 2007). As one of the main substances in isoflavones, genistein can bind to estrogen receptor β and rapidly activate molecular signals of in vivo transduction pathways. We wondered whether phytoestrogens could activate PI3K/Akt signaling pathway and ultimately promote endothelial synthesis of nitric oxide synthase (eNOS) and secretion of NO after they bind to estrogen receptors.

We conducted western-blot in order to detect the target proteins. We found that in genistein-treated rats, the P-Akt and P-eNOS protein expressions were significantly increased. With the increase of the dose of genistein, P-Akt and P-eNOS protein expressions were increased more significantly, while in the rats treated with genistein plus PI3K inhibitor LY294002, the P-Akt and P-eNOS protein expressions were significantly inhibited. This result suggested that the PI3K/Akt/eNOS signal pathway might play an important role for genistein treatment of monocrotaline-induced PAH in rats. This may be one of its main molecular mechanisms. In our determination of NO in lung tissue, we found that the content of NO was increased significantly in genistein treated rat. With the increase of the dose of genistein, a higher content of NO was observed. However, for rats in the group of PI3K inhibitor LY294002, we observed less content of NO, with a comparable NO content as in the PAH group. These results indicated that genistein might increase expression of NO in lung tissue, and this effect could be blocked by a PI3K inhibitor LY294002. Taken together, we believed that PI3K/Akt/eNOS molecular signaling pathway played a role for genistein treatment of PAH in rats.

In conclusion, we confirmed that genistein could reduce pulmonary artery pressure in monocrotaline-induced rat model of PAH, improve the right heart function and ameliorate survival rate. Our study suggested that its mechanism was related with the PI3K/Akt/eNOS signal pathway. Phytoestrogen genistein may become a new and effective drug for patients with PAH.

*Acknowledgements.* This research was funded by Jiangxi Provincial Department of Education (project number: GJJ12101) and by Jiangxi Provincial Department of Science and Technology (project number: 20142BBG70057).

*Conflict of interest:* There is no conflict of interest.

## References

Agarwal R., Shah S.J., Foreman A.J., Glassner C., Bartolome S.D., Safdar Z., Coslet S.L., Anderson A.S. and Gomberg-Maitland M. (2012). Risk assessment in pulmonary hypertension associated with heart failure and preserved ejection fraction. J. Heart Lung Transplant. 31, 467-477.

Chrzan B.G. and Bradford P.G. (2007). Phytoestrogen activate estrogen receptor beta1 and estrogenic responses in human breast and bone cancer cell lines. Mol. Nutr. Food Res. 51, 171-177.

Dubey R.K., Imthum B., Barton M. and Jackson E.K. (2005). Vascular consequences of menopause and hormone therapy: importance of timing of treatment and type of estrogen. Cardiovasc. Res. 66, 295-306.

Falcetti E., Hall S.M., Phillips P.G., Patel J., Morrell N.W., Haworth S.G. and Clapp L.H. (2010). Smooth muscle proliferation and role of the prostacyclin (IP) receptor in idiopathic pulmonary arterial hypertension. Am. J. Respir. Crit. Care Med. 182, 1161-1170.

Fitzpatrick J.L., Mize A.L., Wade C.B., Harris J.A., Shapiro R.A. and Dorsa D.M. (2006). Estrogen mediated neuroprotection against β-amyloid toxicity requires expression of estrogen receptor or and B and activation of the MAPK pathway. J. Neurochem. 82, 674-682.

Galiè N., Palazzini M. and Manes A. (2010). Pulmonary arterial hypertension: from the kingdom of the near-dead to multiple clinical trial meta-analyses. Eur. Heart J. 31, 2080-2086.

Haynes M.P., Li L., Sinha D., Russell K.S., Hisamoto K., Baron R., Collinge M., Sessa W.C. and Bender J.R. (2003). Src kinase mediates phosphatidylinositol 3-kinase/Aktdependent rapid endothelial nitric-oxide synthase activation by estrogen. J. Biol. Chem. 278, 2118-2123.

Hiroki J., Shimokawa H., Mukai Y. and Takeshita A. (2005). Divergent effects of estrogen and nicotine on Rho-kinase expression in human coronary vascular smooth muscle cells. Biochem. Biophys. Res. Commun. 326, 154-159.

Humbert M., Sitbon O., Yaïci A., Montani D., O'Callaghan D.S., Jaïs X., Parent F., Savale L., Natali D., Günther S., Chaouat A., Chabot F., Cordier J.F., Habib G., Gressin V., Jing Z.C., Souza R., Simonneau G. and French Pulmonary Arterial Hypertension Network. (2010). Survival in incident and prevalent cohorts of patients with pulmonary arterial hypertension. Eur. Respir. J. 36, 549-555.

Lahm T., Patel K.M., Crisostomo P.R., Markel T.A., Wang M., Herring C. and Meldrum D.R. (2007). Endogenous estrogen attenuates pulmonary artery vasoreactivity and acute hypoxic pulmonary vasoconstriction: the effects of sex and menstrual cycle. Am. J. Physiol. Endocrinol. Metab. 293, 865-871.

Martin D., Song J., Mark C. and Eyster K. (2008). Understanding the cardiovascular actions of soy isoflavones: potential novel targets for antihypertensive dmg development. Cardiovasc. Hematol. Disord. Drug Targets. 8, 297-312.

Matori H., Umar S., Nadadur R.D., Sharma S., Partow-Navid R., Afkhami M., Amjedi M. and Eghbali M. (2012). Genistein, a soy phytoestrogen, reverses pulmonary hypertension and prevents right heart failure in rats. Hypertension 60, 425-430.

Pugh M.E. and Hemnes A.R. (2010). Pulmonary hypertension in women. Expert Rev. Cardiovasc. Ther. 8, 1549-1558.

Rabinovitch M. (2008). Molecular path genesis of Pulmonary arterial hypertension. J. Clin. Invest. 118, 2372-2379.

Si H.W. and Liu D.M. (2008). Soy phytoestrogen genistein up-regulates the expression of human endothelial oxide synthase and lowers blood pressure in spontaneously hypertensive rats. J. Nutr. 138, 297-304.

Stirone C., Boroujerdi A., Duckles S.P. and Krause D.N. (2005). Estrogen receptor activation of phosphoinositide-3 kinase, akt, and nitric oxide signaling in cerebral blood vessels: rapid and long-term effects. Mol. Pharmacol. 67, 105-113.

Tofovic S.P. (2010). Estrogens and development of pulmonary hypertension Interaction of estradiol metabolism and pulmonary vascular disease. J. Cardiovasc. Pharmacol. 56, 696-708.

Yu X., Rajala R.V., McGinnis J.F., Li F., Anderson R.E., Yan X., Li S., Elias R.V., Knapp R.R., Zhou X. and Cao W. (2004). Involvement of insulin/phosphoinositide 3-kinase/akt signal pathway in 17β-estradiol-mediated neuroprotection. J. Biol. Chem. 279, 13086-13094.

Zhao Y.D., Courtman D.W., Deng Y., Kugathasan L., Zhang Q. and Stewart D.J. (2005). Rescue of monocrotaline-induced pulmonary arterial hypertension using bone marrow-derive endothelial-like progenitor cells: efficacy of combined cell and eNOS gene therapy in established disease. Circ. Res. 96, 442-450.

Accepted April 18, 2016