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Original Article

Effects of total flavonoids of Epimedium on hemodynamics and angiotensin II levels in rats with congestive heart failure via NF-κB

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Abstract: Objective: To investigate the effects of total flavonoids of Epimedium (TFE) on hemodynamics and angiotensin II (Ang II) levels in rats with congestive heart failure (CHF) via NF-κB. Methods: Sixty healthy male rats were divided into blank group, CHF group (CHF model), MET group (CHF model + metoprolol), and TFE group (CHF model + total flavonoids of Epimedium). All rats' general characteristics were observed. The hemodynamics was detected by BL-410 bio-functional experiment system, and myocardial histopathology was detected by hematoxylin and eosin staining. Enzyme-linked immunosorbent assay was used for detecting plasma norepinephrine and Ang II level, and Western blot and reverse transcription polymerase chain reaction was used to detect NF-κB expression. Results: Some rats in CHF group walked slowly. They had bloody secretions, edema of limbs, and appetite loss, and their noses showed cyanosis. These symptoms were improved to varying degrees after drug intervention. Compared with the blank group, left ventricular end diastolic pressure (LVEDP) and heart rate (HR) were increased, while left ventricular systolic pressure (LVSP) and maximal rate of increase/decrease of left ventricular pressure (±dP/dTmax) were decreased in the CHF group (all $P<0.05$). Compared with the CHF group, the HR, LVEDP and LVSP were decreased after drug intervention in the MET group, while ±dp/dtmax were increased (all $P<0.05$). Compared with the MET group, the mentioned indictors were increased in the TFE group but with no significant differences (all $P>0.05$). Intact myocardial cells could be found in the blank group; the columnar transverse striations were clear, and no inflammatory infiltration or myocardial hypertrophy was observed. The myocardial fibers in the CHF group showed obvious apoptosis and elongated; the transverse striations disappeared, and local fibrosis appeared with a lot of inflammatory factors. In the TFE and MET groups, cardiomyocyte hypertrophy and inflammatory infiltration improved (both $P<0.05$). Compared with the blank group, plasma norepinephrine and Ang II levels in CHF group were increased significantly (both $P<0.05$). Compared with the CHF group, plasma norepinephrine and Ang II levels in the TFE and MET groups were decreased (both $P<0.05$). The NF-κBp65 expression in the CHF group was significantly higher than that in the blank group ($P<0.05$). Compared with CHF group, the NF-κBp65 expression in the TFE and MET groups decreased after drug intervention ($P<0.05$). Conclusion: TFE can improve the hemodynamics of rats, increase myocardial oxygen supply, reduce the expression of norepinephrine and Ang II in the plasma of CHF rats, and improve heart function. These changes may be related to the inhibition of NF-κB activity by TFE.

Keywords: Congestive heart failure, Ang II, total flavonoids of Epimedium, NF-κB

Introduction

Congestive heart failure (CHF), also known as heart failure, is the terminal for the development of many heart diseases [1]. In most cases, patients with heart failure are inadequate in cardiac ejection function; their circulating blood volume in the heart is reduced, which is unable to meet the needs of the body's metabolism. In clinic, multiple circulatory disorders occur [2]. According to global big data, nearly 1.8% of people worldwide suffer from CHF, and the incidence of this disease increases with growing age; the high mortality caused by CHF has been a problem for the medical community [3]. Epimedium belongs to the Berberidaceae, which has anti-aging and anti-tumor effects. Total flavonoids of Epimedium (TFE) is a substance extracted from the stems and leaves of Epimedium, which mainly contains icariin and icarisid. Dong et al. found that TFE played a therapeutic role in rats with heart failure by ^{45}Ca transmembrane technology test [4].

In recent years, it has been reported that as the main hormone of renin-angiotensin system, angiotensin II (Ang II) is closely related to the occurrence of CHF [5]. From animal experiments, Ang II can accelerate the strength of cardiomyocyte synthesizing protein ability, leading to cardiomyocyte hypertrophy [6]. It is also found that Ang II does well in vasoconstriction, which results in faster heart rate, proliferation of vascular smooth muscle, and damage to normal hematopoietic function of the heart [7]. However, it is still rarely reported that whether TFE-mediated improvement of the cardiac blood flow is related to Ang II and NF-κB. Therefore, the mechanism among NF-κB signaling pathway, hemodynamics and Ang II were researched by gavage of TFE in a rat model of heart failure.

Materials and methods

Drugs and instruments

Isoproterenol hydrochloride (ISO, SIGMA, USA), metoprolol tartrate (AstraZeneca, USA), total flavonoids of Epimedium (Jiangsu Kanion Pharmaceutical Co. Ltd., China), hematoxylin and eosin stain kit (Shanghai Yiji Industrial Co. Ltd., China), DMEM medium containing fetal bovine serum (Shanghai Sangon Biotech, China), and NF-κBp65 kit (Shanghai Chenyi Bio, China).

Animals and grouping

Sixty male specific-pathogen-free SD rats at 8 weeks of age were selected for the study (300-350 g, purchased from the Test Center of Heilongjiang University of Chinese Medicine). All rats had free access to movability and diet using a pet fountain. They were divided into 4 groups. The rats in the blank group received subcutaneous injection of normal saline, and the model of heart failure wasn't established. The rats in other 3 groups were congestive heart failure model rats: CHF group (CHF model, n=15), MET group (CHF model + metoprolol, 8 mg/kg/day, n=15), and TFE group (CHF + TFE, 200 mg/kg/day, n=15). All rats were regularly fed for 18 weeks. This study was approved by the Animal Experimental Ethics Committee of Hainan Medical University.

Establishment of rat models of heart failure

Before modeling, rats were fed for 2 days to acclimate. Then rats were given subcutaneous injection of ISO (300 mg/kg) at fasting state and then injected again 24 h later. Rats in the blank group were injected with the same volume (300 mg/kg) of normal saline. After 6 weeks, left ventricular ejection fraction (LVEF) was detected by echocardiography. It was regarded as a successful model when LVEF was less than 45%. The diet and activity of rats in each group were observed postoperatively [4].

Hemodynamic test

After the experiment, rats in the 4 groups were anesthetized. The catheter was inserted from the carotid artery into the left ventricle, and the signal was input to the BL-410 bio-functional experiment system (Chengdu Taimeng Technology) through a pressure transducer to detect the left ventricular end diastolic pressure (LVEDP), heart rate (HR), left ventricular systolic pressure (LVSP) and maximal rate of the increase/decrease of left ventricular pressure (±dP/dTmax).

Hematoxylin and eosin staining

After the hemodynamics test, the rats were sacrificed and their left ventricle myocardium was extracted. After fixation, the tissue was dehydrated with ethanol and then embedded in paraffin. The tissue was cut into sections of 5 μm. The sections were blocked with goat serum, stained with hematoxylin for 6-8 min, and restained with eosin solution for 10 s. The myocardial tissue was observed under the microscope.

Plasma norepinephrine and Ang II detection

After the hemodynamic test in rats was completed, the carotid artery blood of rats was extracted. The concentration of norepinephrine was detected by enzyme-linked immunosorbent assay, and Ang II level was detected by radioimmunoassay. The specific operation method was carried out according to the instructions of kits (Shanghai Hengyuan Biotechnology Co. Ltd., China).

NF-κBp65 protein expression detected by Western blot

Myocardial cells (50 μg) were obtained by conventional method. Electrophoresis tank and other components were washed and dried by

Table 1. Primer sequence

Gene	Primer sequence (5'-3')
NF-κBp65	F: 5'-CTGAACCAGGGCATACCTGT-3' R: 5'-GAGAAGTCCATGTCCGCAAT-3'
β-actin	F: 5'-GACTTCAACAGCGACACCC-3' R: 5'-ACCCTGTTGCTGTAGCCAA-3'

baking. Separation gel (12%) and an aqueous layer (1 cm) were prepared. Electrophoresis apparatus was placed for 1 h for coagel, and the spacer gel was prepared and infused in the apparatus. Sample suspension of 50 μg/well was added. Electrophoresis was started at 90 V followed by pouring bromophenol blue into separation gel, and then the voltage was raised to 120 V. Electrophoresis was terminated and the gel was removed. Methanol was put in PVDF membrane for 5-10 s. Filter papers were soaked in transfer buffer within 15 min. PVDF membrane was moved into transfer tank within 10 min in which transfer buffer was supplemented, and then the transfer tank was put in an ice bath at 70 V for 1.5 h. PVDF membrane was taken out from the tank after finishing transferring membrane and washed with tris buffered saline tween (TBST) twice, for 10 min each time. PVDF membrane was placed in sealing solution and sealed in a horizontal shaking table for 2 h. PVDF membrane was then put into a homemade hybridization bag containing primary antibody (Abcam, UK) at 4°C overnight (hybridization solution diluting primary antibody, GAPDH:1:1,000). PVDF membrane was taken out and washed with 1×TBST three times, for 10 min each time. After wash, the membrane was incubated in the corresponding second antibody (KPL, USA) at room temperature for 1.5 h with slight shaking. Then PVDF membrane was washed with TBST three times, for 10 min each time. Solutions A and B in ECL kit (1:1, Shanghai Westang Bio-Tech Co. Ltd., China) were mixed and added in PVDF membrane for about 1 min. Automatic Chemiluminescence Imaging System was employed to detect PVDF membrane.

NF-κBp65 expression detected by reverse transcription polymerase chain reaction

The rats in each group were anesthetized with pentobarbital and sacrificed. The sterile myocardial tissues from the left ventricle were carefully weighed at low temperature (about 100 mg) and ground in liquid nitrogen followed by homogenizing with lysis buffer at low temperature and centrifugation at 2200 r/min for 15 s. The total RNAs were isolated and added with isopropyl alcohol for centrifugation. Afterward, the RNAs were treated with 75% ethanol, centrifuged, dried, and kept at -80°C. The RNAs were then reversely transcribed into cDNAs according to the manufacturer's instructions of the kit. β-actin was used as an internal control. The running parameters of polymerase chain reaction were 60°C for 10 min (pre-denaturation), 95°C for 30 s (denaturation), 72°C for 30 s (annealing), and 95°C for 5 min (extension) for 40 cycles. Each experiment was repeated at least three times, and the relative expression levels of the genes in cardiomyocytes were calculated by $2^{-\Delta\Delta Ct}$ method. See **Table 1**.

Statistical analysis

SPSS22.0 was used for statistical analysis. T-test was adopted for comparison between groups, and the experimental results were expressed as mean ± standard deviation ($\bar{x}$ ± sd). One-way ANOVA was used to compare the results among groups and bonferonni was used to compare the results between two groups. $P<0.05$ was considered to be a statistically significant difference.

Results

General characteristics

Compared with the blank group, some rats in the CHF group walked slowly. They had bloody secretions, edema of limbs, and appetite loss. Their noses showed cyanosis. And these symptoms were improved to varying degrees after 18-week drug intervention.

Hemodynamic test outcome

Compared with the blank group, LVEDP and HR were increased, while LVSP and ±dP/dTmax were decreased in the CHF group (all $P<0.05$). Compared with the CHF group, the HR, LVEDP and LVSP were decreased after drug intervention in the MET group, while ±dp/dtmax were increased (all $P<0.05$). Compared with the MET group, the mentioned indictors were increased in the TFE group but with no significant differences (all $P>0.05$). See **Table 2**.

Table 2. Hemodynamic test outcome

Group	n	HR	LVEDP	LVSP	+dp/dtmax	-dp/dtmax
Blank group	15	400.14±14.32	1.22±0.65	120.17±2.35	3.85±0.08	-3.62±0.03
CHF group	15	453.26±10.39*	10.31±1.25*	104.39±1.79*	2.34±0.05*	-1.98±0.07*
MET group	15	347.39±9.78*,#	5.42±0.91*,#	100.13±1.53*,#	3.35±0.04*,#	-2.85±0.03*,#
TFE group	15	353.59±7.43*,#	5.52±1.09*,#	103.49±1.49*,#	3.39±0.06*,#	-2.75±0.05*,#
F		285.14	207.76	282.23	169.12	297.56
P		<0.001	<0.001	<0.001	<0.001	<0.001

Note: Compared with the blank group, *P<0.05; compared with the CHF group, #P<0.05. LVEDP, left ventricular end diastolic pressure; HR, heart rate; LVSP, left ventricular systolic pressure; ±dP/dTmax, maximal rate of the increase/decrease of left ventricular pressure; CHF, congestive heart failure; MET, metoprolol; TFE, total flavonoids of Epimedium.

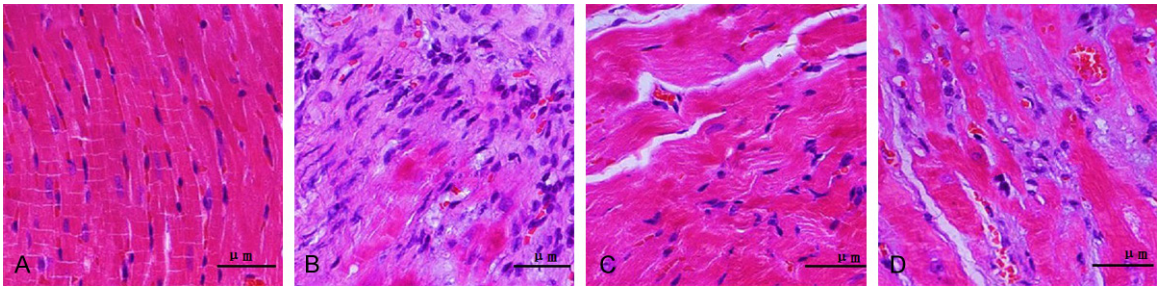

Figure 1. Histopathological results of the heart (×400). A. Blank group; B. CHF group; C. MET group; D. TFE group. CHF, congestive heart failure; MET, metoprolol; TFE, total flavonoids of Epimedium.

Table 3. Adrenal hormone and Ang II levels outcome

Group	n	Adrenal hormone (ng/mL)	Ang II (pg/mL)
Blank group	15	33.25±5.46	271.34±53.29
CHF group	15	82.19±6.38*	663.21±32.28*
MET group	15	68.43±14.39*,#	517.33±43.21*,#
TFE group	15	62.15±5.37*,#,&	505.16±61.23*,#,&
F		83.19	165.91
P		<0.001	<0.001

Note: Compared with the blank group, *P<0.05; compared with the CHF group, #P<0.05; compared with the MET group, &P<0.05. CHF, congestive heart failure; MET, metoprolol; TFE, total flavonoids of Epimedium; Ang II, angiotensin II.

Histopathological results of the heart

Intact myocardial cells could be found in the blank group; the columnar transverse striations were clear, and no inflammatory infiltration was observed. The myocardial fibers in the CHF group showed apoptosis and elongated; the transverse striations disappeared, and local fibrosis appeared with a lot of inflammatory factors. In the TFE and MET groups, cardiomyocyte hypertrophy and inflammatory infiltration were improved. See **Figure 1**.

Norepinephrine and Ang II level outcome

Compared with the blank group, norepinephrine and Ang II level in CHF group were increased significantly (both P<0.05). Compared with the CHF group, the expression levels of norepinephrine and Ang II in the TFE and MET groups were decreased in different degrees (both P<0.05). The above indicators in the TFE group were lower than those in the MET group (both P<0.05). See **Table 3**.

NF-κBp65 protein expression

NF-κBp65 protein expression in the CHF group was significantly higher than that in the blank group (P<0.05). Compared with the CHF group, NF-κBp65 protein expression in the TFE group and MET group was decreased after drug intervention (P<0.05). There was no difference between the MET group and TFE group (P>0.05). See **Figure 2**.

NF-κBp65 mRNA expression

The NF-κBp65 mRNA expression in the CHF group was significantly higher than that in the blank group (P<0.05). Compared with the CHF group, NF-κBp65 mRNA expression in the TFE and MET groups was decreased after drug

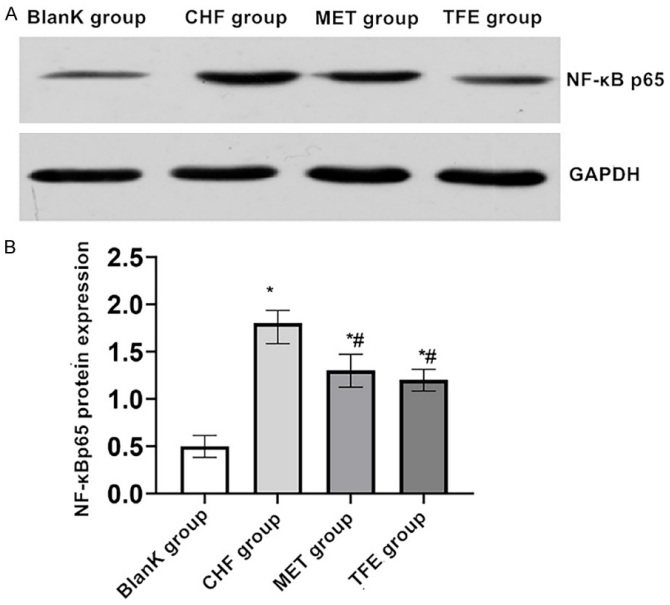

Figure 2. NF-κBp65 protein expression. A. NF-κBp65 protein bands; B. NF-κBp65 protein expression. Compared with the blank group, *P<0.05; compared with the CHF group, #P<0.05. CHF, congestive heart failure; MET, metoprolol; TFE, total flavonoids of Epimedium.

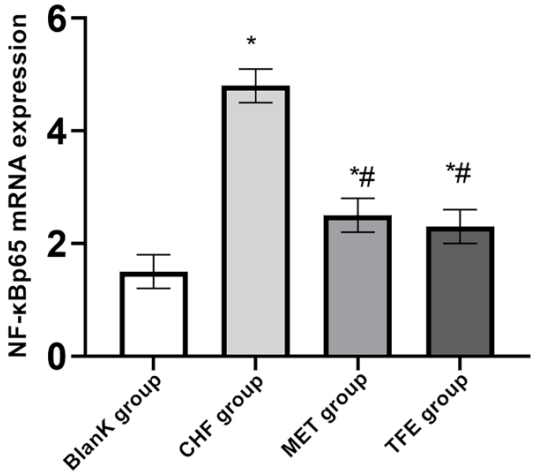

Figure 3. NF-κBp65 mRNA expression. Compared with the blank group, *P<0.05; compared with the CHF group, #P<0.05. CHF, congestive heart failure; MET, metoprolol; TFE, total flavonoids of Epimedium.

intervention (P<0.05). There was no difference between the MET group and TFE group (P> 0.05). See **Figure 3**.

Discussion

Rat models of heart failure can be established by a variety of methods. In this study, heart failure models in rats were established after ISO injection. By observing the characteristics of rat heart load and heart rate and using pathological specimens, it is beneficial to observe the process of heart failure in rats.

In this study, we found that after giving TFE, rats with heart failure moved less after 6 weeks, and the groups with drug intervention showed different degrees of improvement. Rats in each group were tested for hemodynamics by BL-410 bio-functional experiment system. It was found that LVEDP and HR in rats with heart failure were increased, while LVSP and ±dP/dTmax were decreased. Compared with the blank group, the HR, LVEDP and LVSP were decreased after drug intervention in the MET and TFE groups, while ±dp/dtmax were increased. The HR and LVEDP in the TFE group were a little higher than those in the MET group, but without significant difference. The above results revealed that after drug intervention, the heart function was improved and LVSP was increased in heart failure rats. A large number of studies have shown that patients with heart failure are often accompanied by changes in hemodynamics of the heart, such as HR, LVEDP, LVSP, and ±dp/dtmax levels [8, 9]. In this study, LVEDP and HR were increased when myocardial tissue was damaged and heart function was impaired. After TFE intervention, the above indicators were decreased, indicating that TFE could timely relieve LVEDP, HR, maintain heart function and protect myocardial tissues when myocardial tissue was damaged. ±dp/dtmax is commonly used to evaluate myocardial contraction and diastole and show the ventricular wall tension. +dp/dtmax is used to indicate myocardial contractility, and the increase or steadiness of -dp/dtmax represents increased myocardial contraction and improved diastolic function [10, 11]. A lot of studies have shown that metoprolol can inhibit the adrenal medullary system, reduce aldosterone synthesis, vicious circle of oxidative stress and myocardial cell apoptosis, as well as delay heart failure. In this study, we found that dp/dtmx increased in rats with heart failure treated with TFE and MET, indicating that both TFE and MET could maintain the decrease of dp/dtmax caused by myocardial ischemia to a certain extent, and the increased

LVSP improved myocardial contraction and diastolic function, which was beneficial to improve myocardial efficiency [12-14].

In this study, the plasma norepinephrine and Ang II levels of rats with heart failure were increased, and then decreased after TFE treatment. Norepinephrine and Ang II are indicators of ischemia and hormones, which can lead to abnormal metabolism of heart failure [14]. It has been found that the occurrence of heart failure is closely related to cardiac hypertrophy [15]. Ang II is a key factor to activate cardiac hypertrophy. It can lead to apoptosis of cardiomyocytes, cardiac hypertrophy and heart failure, by activating MARK signal pathway [15]. In this study, the expression levels of adrenal hormone and Ang II in the TFE Group were decreased in different degrees compared to the CHF group, which indicates that TFE had the function of antioxidation as well as improving myocardial hemodynamics. Liu et al. reported that the main components of TFE could reduce the plasma concentration of adrenal hormone and Ang II in CHF rats and delay heart failure [16], which was similar to the results obtained by Yang et al. [17].

NF-κB, an important transcription factor, plays an important role in inflammatory response and pathological process. In this study, we found that the expression of NF-κB decreased in the myocardium of the rats with heart failure when treated with TFE, indicating an inhibitory function of TFE on NF-κB. Wang et al. found that the NF-κB was seldom expressed in normal myocardial tissue [18]; instead, on exogenous stimulation, NF-κB would transfer from cell lipids to the nucleus, thereby aggravating inflammatory reaction of myocardial tissue. In the study of Tian et al., the expression of NF-κB in myocardial tissue was inhibited by TFE [19]. It might be related to the anti-inflammatory effect of TFE, the protection of myocardial tissue and the improvement of cardiac function. In the study by Georgakopoulos et al. [20, 21], after metoprolol was used to treat heart failure, it was found that NF-κB-56 in serum was significantly reduced and heart function was improved.

In conclusion, TFE can improve the hemodynamics of rats, increase myocardial oxygen supply, reduce the expressions of norepinephrine and Ang II of rats with heart failure, and improve the heart function. These effects may be related to the inhibition of NF-κB activity by TFE.

Disclosure of conflict of interest

None.

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References

[1] Suthahar N, Meijers WC, Sillje HHW, Ho JE, Liu FT and de Boer RA. Galectin-3 activation and inhibition in heart failure and cardiovascular disease: an update. Theranostics 2018; 8: 593-609.

[2] Oellgaard J, Gaede P, Rossing P, Rorth R, Kober L, Parving HH and Pedersen O. Reduced risk of heart failure with intensified multifactorial intervention in individuals with type 2 diabetes and microalbuminuria: 21 years of follow-up in the randomised Steno-2 study. Diabetologia 2018; 61: 1724-1733.

[3] Dawwas GK, Smith SM and Park H. Risk of heart failure hospitalization among users of dipeptidyl peptidase-4 inhibitors compared to glucagon-like peptide-1 receptor agonists. Cardiovasc Diabetol 2018; 17: 102.

[4] Dong JJ, Yan GQ, Chen SL and Lu Q. Effects of total flavonoids of Epimedium on myocardial cells and serum immunoregulatory factors in VMC mice. J Sichuan Tradit Chin Med 2017; 11: 56-58.

[5] Zhou YH, Zhou YH, Yang GD and Bai DN. Angiotensin H promotes collagen synthesis in cardiac fibroblast via miR-21. Chin Heart J 2017; 7: 40-43.

[6] Chen L, Li Q, Lei L and Li T. Dioscin ameliorates cardiac hypertrophy through inhibition of the MAPK and Akt/GSK3 beta/mTOR pathways. Life Sci 2018; 209: 420-429.

[7] Jeong J, Lee J, Lim J, Cho S, An S, Lee M, Yoon N, Seo M, Lim S and Park S. Soluble RAGE attenuates AngII-induced endothelial hyperpermeability by disrupting HMGB1-mediated crosstalk between AT1R and RAGE. Exp Mol Med 2019; 51: 113.

[8] Li X, Wang MM, Gu F, Gao YQ, Zhang BY, Zhang CY and Guo MJ. Research progress of Herba Epimedii and its main components in regulating AMPK pathway in prevention and treatment of heart failure. Shanghai J Tradit Chin Medi 2019; 53: 106-110.

[9] Li Y, Zhu Q, Guo Y, Guo R, Zhao FM, Li W and Bian HM. Effects of ligustrazine structural modification product liguzinediol on hemodynamics of chronic heart failure model rats induced by adriamycin. Zhongguo Yaofang 2019; 30: 21-26.

[10] Erkelens CD, van der Wal HH, de Jong BM, Elting JW, Renken R, Gerritsen M, van Laar PJ, van Deursen VM, van der Meer P, van Veldhuisen DJ, Voors AA and Luijckx GJ. Dynamics of cerebral blood flow in patients with mild non-ischaemic heart failure. Eur J Heart Fail 2017; 19: 261-268.

[11] Jorgenson CC, Chase SC, Olson LJ and Johnson BD. Assessment of thoracic blood volume by computerized tomography in patients with heart failure and periodic breathing. J Card Fail 2018; 24: 479-483.

[12] Ji YQ, Luo J, Lu LL and Zhang QF. Study on the pharmacodynamic basis and mechanism of the active components of traditional Chinese medicine against heart failure. Zhongguo Yaofang 2019; 30: 145-150.

[13] Li J, Deng LL, Zhou ZY, Yuan D, Zhang CC and Wang T. Protective effect of total saponins of panax notoginseng combined with total flavonoids of epimedium on D-galactose-incuced senescence of H9c2 cell. Zhongguo Zhongyao Zazhi 2017; 42: 555-561.

[14] Jia EJ. Changes of serum ang II, ALD and NE in patients with heart failure complicated with pulmonary infection and their relationship with cardiac function grading. Chin J Integr Med Cardio-/Cerebrovascuiar Dis 2018; 16: 980-982.

[15] Astorino TA, Sherrick S, Mariscal M, Jimenez VC, Stetson K and Courtney D. No effect of meal intake on physiological or perceptual responses to self-selected high intensity interval exercise (HIIE). Biol Sport 2019; 36: 225-231.

[16] Liu XY, Liao HH, Feng H, Zhang N, Yang JJ, Li WJ, Chen S, Deng W and Tang QZ. Icariside II attenuates cardiac remodeling via AMPK alpha 2/mTORC1 in vivo and in vitro. J Pharmacol Sci 2018; 138: 38-45.

[17] Yang L, Peng C, Xia J, Zhang W, Tian L, Tian Y, Yang X and Cao Y. Effects of icariside II ameliorates diabetic cardiomyopathy in streptozotocin-induced diabetic rats by activating Akt/NOS/NF-kappa B signaling. Mol Med Rep 2018; 17: 4099-4105.

[18] Wang Y, Wang L, Li JH, Zhao HW and Zhang FZ. Morphine alleviates myocardial ischemia/reperfusion injury in rats by inhibiting TLR4/NF-kappa B signaling pathway. Eur Rev Med Pharmacol Sci 2019; 23: 8616-8624.

[19] Tian C, Chen X, Chang Y, Wang R, Ning J, Cui C and Liu M. The regulatory effect of flavonoids extracted from abutilon theophrasti leaves on gene expression in LPS-induced ALI mice via the NF-kappa B and MAPK signaling pathways. Pharm Biol 2019; 57: 514-518.

[20] Georgakopoulos P, Kyriakidis M, Perpinia A, Karavidas A, Zimeras S, Mamalis N, Kouvela M and Charpidou A. The role of metoprolol and enalapril in the prevention of doxorubicin-induced cardiotoxicity in lymphoma patients. Anticancer Res 2019; 39: 5703-5707.

[21] Mishra T, Ahmed A, Shokr M, Rashed A, Yassin AS and Kottam A. Early-onset bilateral severe valvular regurgitation after mediastinal radiotherapy in hodgkin lymphoma survivors: should we screen prior to 10 years after mediastinal radiotherapy? Ochsner J 2019; 19: 252-255.