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Therapeutical Efficacy of Bee Venom Compound Preparation Bao Yuan Ling on Rats with Chronic Heart Failure

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Abstract: Objective to explore the influence and possible mechanism of bee venom compound preparation Bao Yuan Ling on chronic heart failure with different dosages (1.00, 1.25, 1.50, 1.75 and 2.00 mg kg^{-1}) the results of this study provided important experimental basis for the clinical application of heart failure. The effects of Bao Yuan Ling on model rats with chronic heart failure induced by doxorubicine were explored and measured using the results of serum physiological indexes, organ coefficient and pathological sections of the apex part of heart. Compared with model group, heart failure related indexes level of BNP, MYO/MB, CK-MB and MDA in serum of BYL groups were significantly decreased while TNF-α and GSH-Px increased significantly ($p<0.05$). No significant organ coefficient changes were induced by the treatment with BYL while dexrazoxane revealed some myocardial hypertrophy effects on heart. Histopathology examination showed changes at myocardial cell level (intercellular substance fibrosis, inflammation infiltration and cell stained) of rats treated with BYL and dexrazoxane at week 6. Moreover, drooping vigor and dilute defecation were relieved in the rats of BYL groups compared with model group. BYL inhibited the progress of chronic heart failure effectively which are superior to the clinic drug dexrazoxane with the optimal dose of 1.50 mg kg^{-1}.

Key words: Bee venom compound preparation, doxorubicine, chronic heart failure, dexrazoxane, myocardial

INTRODUCTION

Heart failure is an serious cardiovascular disease caused by impaired myocardium for multiple reasons so that cardiac structure and functions are influenced and cardiac output are not able to meet the regular need. With high morbidity, its 5 years survival rate is similar to that of malignant tumor, specific antidote can hardly be found to cure heart failure at present (Hunt *et al*., 2005).

Bee venom has aromatic flavour and complex composition, its main bioactive substance is polypeptide in which melittin, MCD peptide, cadiopep and apamin play an important role in improving cardiac functions and regulation (Habermann, 1972). Melittin reduces peripheral vascular pressure by destroying the structure of mastocytes to release histamine and stimulate brain blood vessels, increases coronary blood flow there by ameliorating blood pressure, arrhythmia and heart failure. MCD peptide lowers the blood pressure by stimulating mastocytes degranulation to release histamine. Cadiopep and apamin both have anti-arrhythmic effects. Besides phospholipase A_2 of bee venom also release histamine by producing soft haemolysis phosphatide. Previous studies have proved clinically that bee venom possesses diverse biological and pharmacological properties which has been demonstrated in treating pathological conditions including hypertension, atherosclerosis, arthritis, pain, tumors, arrhythmia and skin diseases (Yalcin and Savci, 2007; Lee *et al*., 2010; Son *et al*., 2007; Kwon *et al*., 2005; Putz *et al*., 2006).

Bao Yuan Ling (Shenfeng; Fujian, China) is a bee venom compound preparation together with different bee products and traditional Chinese herb. It was clinically proved that Bao Yuan Ling had therapic functions on heart failure. Bee venom contains a variety of active substances with efficacy on cardiovascular system (Thomas and Hiley, 1988; Gromova *et al*., 1992; Son *et al*., 2007) but its main mechanism is still unknown, morphology and the myocardial pathology alterations after treatment are rarely reported. The objective of the present study is to explore effects and possible mechanism of Bao Yuan Ling on inhibiting heart failure process, recovering cardiac functions and protecting the heart by intragastric administration on model rats induced by doxorubicine and evaluating related serum biochemical indexes, organ coefficient (HW/BW) and myocardial

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pathology. The study will contribute to the actual knowledge of bee venom compound preparation Bao Yuan Ling and provide new insights on the clinical role of venom production.

MATERIALS AND METHODS

Animals and doses: Male 120 SD rats (mean body weight 60±5 g, provided by Slack laboratory animal Co., Ltd., Shanghai) were randomized divided into eight groups including control group, model group, 5 BYL groups (1.00, 1.25, 1.50, 1.75 and 2.00 mg kg^{-1}) and dexrazoxane group for treatment after 1 week (n = 15). All the rats except control group were administered intraperitoneal injection with doxorubicine (Danxi; Shanghai, China) at the dose of 4 mg kg^{-1} disposably once a week for 6 weeks to induce chronic heart failure, meanwhile administrated with different drugs: dexrazoxane group was pre-administered dexrazoxane (Aosaikang; Jiangsu, China) intraperitoneally with the dose of 40 mg kg^{-1} 30 min before doxorubicine; 5 BYL groups were administered by lavage with Bao Yuan Ling from week 3 at the doses of 1.00, 1.25, 1.50, 1.75 and 2.00 mg kg^{-1} every 2 days, respectively control group, together with model group was administered by lavage equally with saline. All the animals were controlled under the conditions of temperature 20~22°C, relative humidity 30~35% and 12 h light-dark cycle. The animals were fed with a standard chow and tap water was supplied *ad libitum*.

Experimental design: Serum heart failure related indexes including Brain Natriuretic Peptide (BNP), Creatine Kinase MB isoenzyme (CK-MB), Myoglobin (MYO/MB), Troponin I (Tn-I), Malondialchehyche (MDA), Tumer Necrosis Factor α (TNF-α) and Glutathione Peroxidase (GSH-Px) were measured at weeks 0, 1, 2, 3, 4, 5 and 6 after the treatment; organ coefficient (HW/BW) were measured and pathological examination was administrated at the end of research. After that, all the animals received an overdose of pentobarbital sodium for the measurement of organ coefficient (HW/BW) samples of heart were collected for H.E staining after the rats were sacrificed. All animal studies were conducted according to the regulations for the use and care of experimental animals and approved by the Ethics Committee of Fujian Agriculture and Forestry University.

Measurement

Animal conditions: Continuous observe and record general conditions of the rats including body weight, food consumption, activities, reactions, lips color and fur.

Hematology indexes: The blood was collected from the suborbital vein each week into heparinized tubes for treatment and immediately centrifuged at 3000 rpm for 20 min. The plasma was transferred into clean tubes and stored at -80°C until analysis according to the instructions of Rat BNP, CK-MB, MYO/MB, Tn-I, MDA, TNF-α and GSH-Px ELISA kit (Sangon; Shanghai, China).

Organ coefficient: At the end of the treatment, all the rats received an overdose of pentobarbital sodium for the measurement of Heart Weight (HW), Body Weight (BW) and organ coefficient (HW/BW).

Histopathology examination: Apex part of the hearts were fixed in 4% paraformaldehyde, infiltrated in paraffin and sectioned (Yidi; Zhejiang, China). Sections (3~5 μm) were deparaffinized, rehydrated and then stained with hematoxylin and eosin, histopathology was examined by B203LED light microscopy (Aote; Chongqing, China) in a blinded way.

Statistical analysis: All the data were given as Mean±SD ($\bar{x} \pm S$) and analyzed with SPSS19.0 using ANOVA. Compared with model group, $p<0.05$ was considered as the level of significance.

RESULTS AND DISCUSSION

Animal conditions: Activities of rats in model group decreased from week 3 with drooping vigor, emotioal lability, rough fur, cyanosis lips, dilute defecation, less food consumption, abdominal dropsy, abdominal dropsy and sluggish behavior, necroscopic examination revealed serious ascites, swelling and cyanosis liver. Activities and food consumption of BYL medium dose group (1.50 mg kg^{-1}) decreased in week 2 but recovered from week 3 with increased body weight, healthy lips color and no ascites. While rats of dexrazoxane and BYL high dose group (1.75, 2.00 mg kg^{-1}) had the similar symptoms as model group including purple lips and messy fur but the former gradually emaciated from week 3 with less activities and high mortality. All the body weight of control and BYL groups increased significantly at the end of the experiment, whereas model and dexrazoxane group lost flesh as time passed but due to the serious ascites of model group, no significant difference was observed ($p>0.05$).

Hematology indexes

BNP: Compared with model group, BNP level in BYL medium dose group (1.50 mg kg^{-1}) decreased significantly

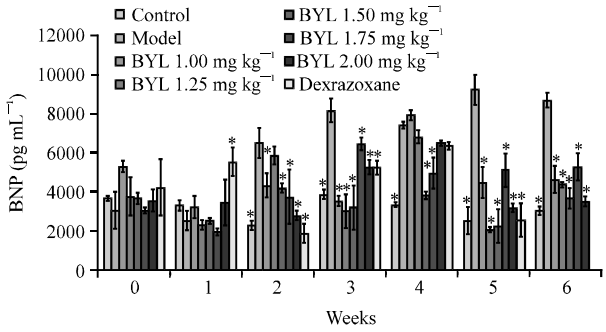

Fig. 1: BNP level of rats during the administration of BYL and dexrazoxane for 6 weeks ($\bar{x} \pm S$, n = 15) compared with model group: *p<0.05

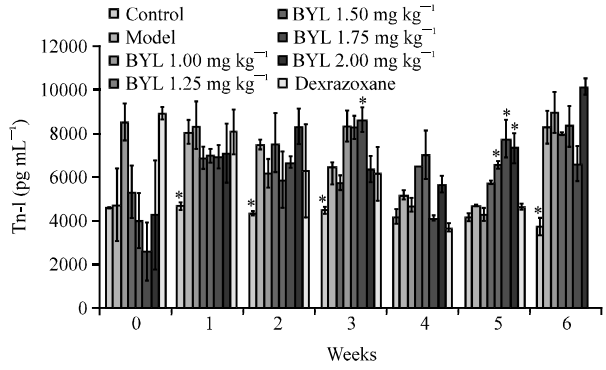

Fig. 2: Tn-I level of rats during the administration of BYL and dexrazoxane for 6 weeks ($\bar{x} \pm S$, n = 15) compared with model group: *p<0.05

(p<0.05). Model group was significantly different from control group from week 2 (p<0.05) and the former increased with a high level until the end (Fig. 1).

Tn-I: Model group increased significantly from week 1 compared with control group (p<0.05) and then decreased gradually. Rats treated with BYL and dexrazoxane did not show significant differences compared with model group (p>0.05) (Fig. 2).

MYO/MB: Compared with control group, model group increased significantly (p<0.05); BYL high dose group (2.00 mg kg^{-1}) decreased from week 3 which was not significant different from model group until week 6 (Fig. 3).

CK-MB: Model group was significantly different from control group from week 2 until the end (p<0.05); all the groups differed from model group on week 3 in which BYL low dose group (1.25 mg kg^{-1}) kept the difference until the end (Fig. 4).

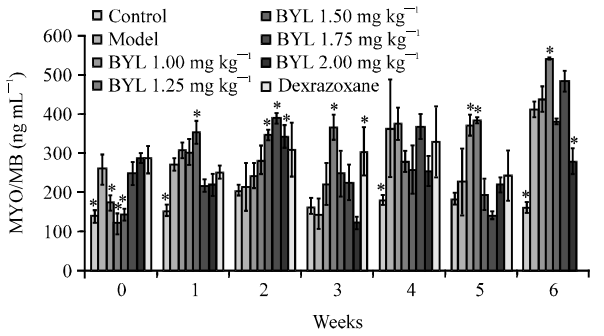

Fig. 3: MYO/MB level of rats during the administration of BYL and dexrazoxane for 6 weeks ($\bar{x} \pm S$, n = 15) compared with model group: *p<0.05

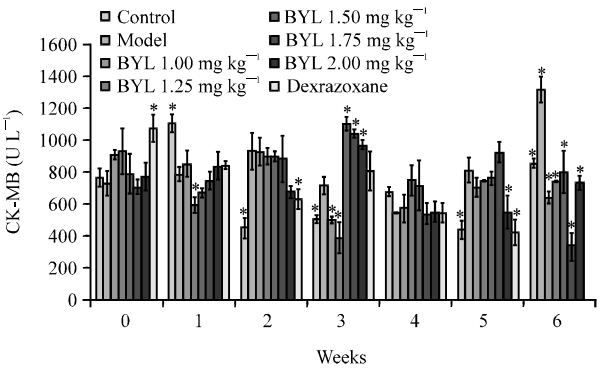

Fig. 4: CK-MB level of rats during the administration of BYL and dexrazoxane for 6 weeks ($\bar{x} \pm S$, n = 15) compared with model group: *p<0.05

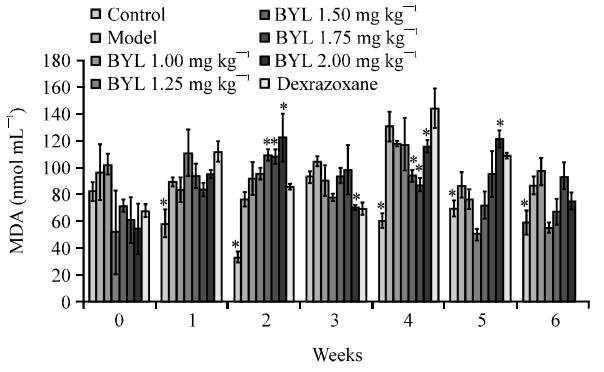

Fig. 5: MDA level of rats during the administration of BYL and dexrazoxane for 6 weeks ($\bar{x} \pm S$, n = 15) compared with model group: *p<0.05

MDA: Model group was significantly different from control group. Medium and high dose of BYL (1.50, 1.75 mg kg^{-1}) decreased MDA level significantly from week 4 compared with model group (p<0.05) (Fig. 5).

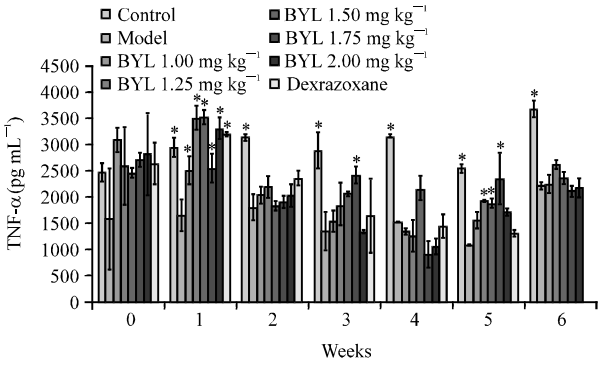

Fig. 6: TNF-α level of rats during the administration of BYL and dexrazoxane for 6 weeks ($\bar{x} \pm S$, n = 15) compared with model group: *p<0.05

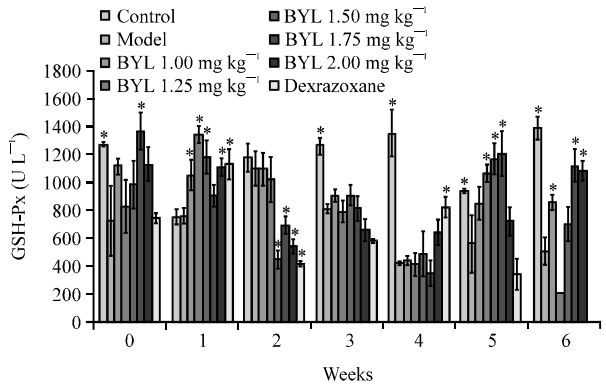

Fig. 7: GSH-Px level of rats during the administration of BYL and dexrazoxane for 6 weeks ($\bar{x} \pm S$, n = 15) compared with model group: *p<0.05

TNF-α: Compared with model group, control group increased significantly (p<0.05), levels of BYL groups were higher than model group (Fig. 6).

GSH-Px: Control group was significantly higher than model group (p<0.05), BYL groups decreased significantly from week 2 and then increased gradually while model group decreased significantly from weeks 3-6 (Fig. 7).

Organ coefficient: Body weight of the rats of model group decreased rapidly but due to serious ascites, the value increased, so that no significant difference was observed (p>0.05) while dexrazoxane group increased significantly compared with model group (p<0.05) (Fig. 8).

Histopathology examination:

- Control group (Fig. 9A): clear cells and fiber structure, complete shape, arranged uniformly and neatly, clear grain, uniform size of cells

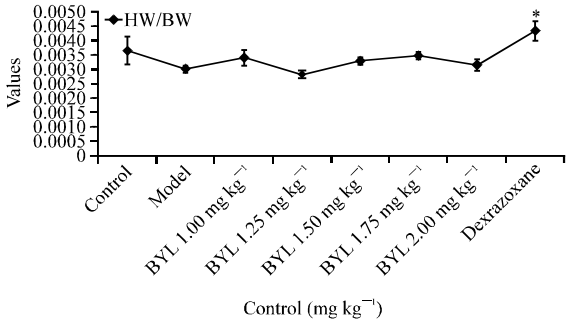

Fig. 8: Organ coefficient of rats after the administration of BYL and dexrazoxane for 6 weeks ($\bar{x} \pm S$, n = 15) compared with model group: *p<0.05

- Model group (Fig. 9B): cells damaged without complete shape, blur edge, disordered arrangement of cardiac fiber, grain fractured and dissolved, hyperemia and edema of mesenchyme, a large number of inflammatory cells infiltration
- BYL 1.00 mg kg^{-1} group (Fig. 9C): interstitial fibrosis with some inflammatory cells infiltration, smaller cells with low density, shallow stained, a small amount of collagen fibers
- BYL 1.25 mg kg^{-1} group (Fig. 9D): interstitial fibrosis with some inflammatory cells infiltration, smaller and less cells, darker staned
- BYL 1.50 mg kg^{-1} group (Fig. 9E): tight arranged cells, slight interstitial fibrosis, some inflammatory cells infiltration, larger cells with high density, dark stained
- BYL 1.75 mg kg^{-1} group (Fig. 9F): interstitial fibrosis, dispersed cells and fiber, clear and complete cells but with low density
- BYL 2.00 mg kg^{-1} group (Fig. 9G): tight arranged intercellular substance, clear stained, complete edge but low density, some inflammatory infiltration
- Dexrazoxane group (Fig. 9H): dispersed arranged and dark stained cells, interstitial fibrosis, inflammatory infiltration

The results showed that heart failure process positively correlated with doxorubicin accumulation amount, early symptoms produced by doxorubicin chemotherapy may be associated with side effects of cardiac toxic. Later symptoms of cardiac insufficiency may due to the accumulation toxicity resulted in myocardial damage which causes heart failure including ascites, hepatomegaly and other symptoms (Ramirez-Exposito and Martinez-Martos, 2008; Landmesser and Drexler, 2007). BNP, Tn-I, MYO/MB and CK-MB are commonly used clinical heart failure mark indexes (Arehan and Toiler, 2008; Kawabata *et al*., 2007).

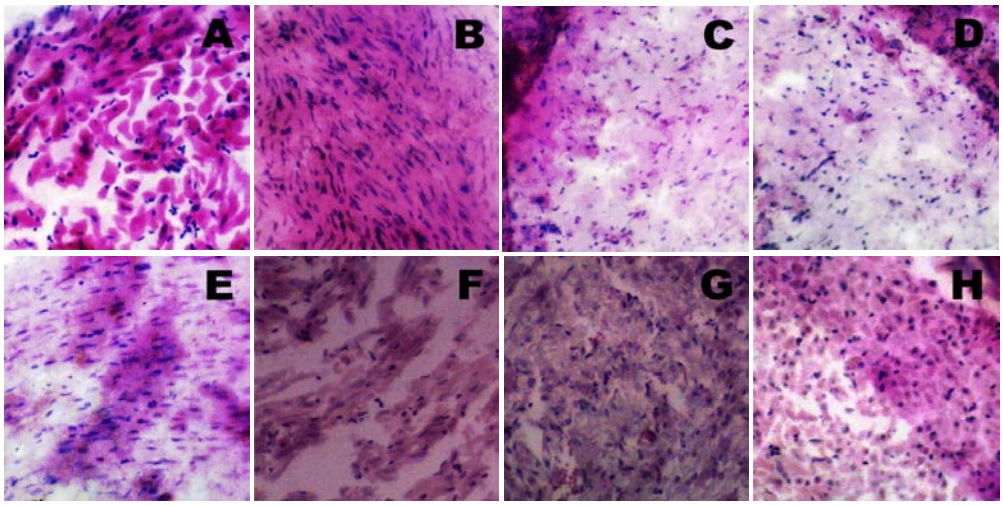

Fig. 9: Comparison of heart Histopathology in week 6: A) control group; B) model group; C) BYL 1.00 mg kg^{-1} group; D) BYL 1.25 mg kg^{-1} group; E) BYL 1.50 mg kg^{-1} group; F) BYL 1.75 mg kg^{-1} group; G) BYL 2.00 mg kg^{-1} group; H) dexrazoxane group (H.E x100)

MDA is the product of oxidation reaction, medium and high dose of Bao Yuan Ling (1.50, 1.75 mg kg^{-1}) significantly inhibited oxidation products; TNF-α induces hemorrhagic necrosis of various tumors, GSH-Px eliminates lipid peroxide, content of these two indexes in control group increased significantly compared with model group and BYL groups but BYL groups increased gradually while model group was just the opposite. Data of dexrazoxane group were not collected at week 6 because the rats all died which indicated that dexrazoxane had high accumulated toxicity and side effect during the research.

The previous studies based on bee venom showed that bee venom concentration dependent increased mice cardiac functions to some extent and reduced heart rate and serum BNP level, the reason might be associated with stimulating blood vessels and thus enhance cardiac functions. Bao Yuan Ling is natural composed preparation made by bee venom and traditional Chinese herb with remarkable therapeutic effects on patients with heart failure clinically proved. With median lethal dose (LD_{50}) 20.54 mg kg^{-1}, it increased heart throb mediated by β receptor, enhanced cardiac shrinkage capacity and had positive inotropic effect on myocardial contraction tension *in vitro*. In this study, BYL decreased content of BNP and MDA significantly, inhibited process of heart failure and removed oxygen free radicals with low side effects and stable persistent which was superior to common clinical drug dexrazoxane. Meanwhile, it has functions including improving immunity, facilitating synthesis and proliferation of DNA in myocardial cells, retarding oxidative stress and cell apoptosis, adjusting acetone dehydrogenase kinase activities of mitochondria, promoting compensation of energy metabolism, etc. In addition, Bao Yuan Ling has inhibitory functions on Lewis lung cancer, S_{180} and H_{22} bearing cancer, effectively improved the living condition of model animals, inhibited proliferation of tumor cells, prolonged the survival time and significantly enhanced the immunity and response of model animals by inhibiting tumor angiogenesis, destroying the nucleus and organelles of tumor cells, suppressing related genes of mitochondrial respiratory chain and tumor proliferation with the dose of 2.0 mg kg^{-1} effectively improved the indexes of spleen and thymus to normal levels. Apitherapy is the characteristic of traditional Chinese medicine which has been gradually recognized by the researchers all over the world. Combining the advantages of traditional Chinese medicine with apitherapy, Bao Yuan Ling avoided the disadvantages of traditional medical with natural formula and low side effects, opened up a new way for clinic treatment.

CONCLUSION

Researchers concluded that bee venom compound preparation Bao Yuan Ling reduced heart failure process efficiently, protected the heart from myocardial cell injury induced by doxorubicin. Further study of the mechanism involved in inhibition of oxidative stress and β receptor mediated cardiac effections of bee venom and its preparations could provide new strategies for prevention and control of myocardial damage and heart failure.

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