

**Protective effect of Esculetin on myocardial injury induced by doxorubicin**FAN XU<sup>2</sup>, XIAO LI<sup>1</sup>, XIAOLEI YU<sup>2\*</sup>Departments of <sup>1</sup>Radiology and <sup>2</sup>Oncology , Affiliated Hospital of Chengde Medical College**Abstract**

**Objective:**To study the protective effect of Esculetin on myocardial injury induced by doxorubicin and explore the mechanism of doxorubicin on myocardial injury in rats.

**Methods:**Doxorubicin(2.5mg/kg) was injected intraperitoneally every other day for 6 times to establish a rat model of myocardial injury.The effects of different doses of Esculetin (10mg/kg&20mg/kg) on serum myocardial enzymes and myocardial tissue were observed.

**Results:**Doxorubicin significantly increased serum myocardial enzymes and the content of myocardial interstitial collagen fibers in mice.Different doses of Esculetin could reduce myocardial injury in a dose-dependent manner.

**Conclusion:** Esculetin has a protective effect on myocardial injury induced by doxorubicin.

*Key words: Esculetin, Doxorubicin, Myocardial injury, Collagen fiber*

**Introduction**

Doxorubicin is the representative drug of anthracycline antitumor antibiotics.It is the most commonly used cell cycle nonspecific chemotherapy drug in clinic.It is used to treat a variety of malignant tumors.It is an important first-line anticancer drug.<sup>1</sup> Although chemotherapy plays an irreplaceable role in tumor,its toxic and side effects should also be paid attention to.Studies have shown that long-term use of doxorubicin may lead to the accumulation of cardiotoxicity.<sup>2</sup> Mild cases are abnormal ECG and myocardial damage.Severe cases may have heart failure or even death,with an incidence of 10%-20%. <sup>3</sup> It has been reported

that the cardiotoxicity of doxorubicin is related to the production of a large number of free radicals and lipid peroxidation.Esculetin(ESC) is the main active component of the traditional Chinese medicine Chenpi,which can scavenge oxygen free radicals and protect cells from the damage caused by peroxide.In this study,the myocardial injury model induced by doxorubicin was used to evaluate the protective effect of Esculetin,so as to provide a basis for further expanding the clinical application of Esculetin.

**Materials and Methods****1. Rats and materials**

Doxorubicin hydrochloride injection was

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purchased from Shanxi Pude Pharmaceutical Co.,Ltd., Esculetin was purchased from Beijing Kangtai Heyuan Biotechnology Co.,Ltd.,Masson staining kit was purchased from Beijing solabao Biotechnology Co.,Ltd.,lactate dehydrogenase(LDH) kit, creatinine kinase(CK) kit, MB isoenzyme of creatine kinase(CK-MB) kits were provided by the laboratory department of our hospital. Forty male SD rats (weighing 220-250g) were purchased from Beijing Kangtai Heyuan biological company (license No.: scxk Jing 2012-0001).

2. Experimental Protocols

The rats were randomly divided into five groups:control group, DOX group, ESC(low dose)+DOX group, ESC(high dose)+DOX group (n=5 per group). Rats in DOX treatment group were intra-peritoneally injected with DOX(dissolved in normal saline,2.5mg/kg every other day, 6 times in total). All rats were gavaged with distilled water or ESC (dissolved in distilled water, high dose:20 mg/kg,low dose:

10 mg/kg) 15 days before DOX.After 10 days of the final administration of DOX, The rats were killed and cardiac tissue and blood samples were collected for histopathological examination.

3.Statistical Analysis

SPSS 25.0 statistical software was used for statistical analysis.All experimental data were expressed as mean±standard deviation. The five groups of measurement data were compared by one-way ANOVA, and the difference was statistically significant( $P<0.05$ ).

Results

1. Effect of ESC on myocardial enzymes after DOX interference in rats

Compared with the control group, myocardial enzymes in DOX group increased significantly. Compared with DOX group, all indexes of myocardial enzymes in ESC low and high dose+DOX group decreased significantly, indicating that ESC reduced the damage of DOX to myocardial tissue (Table 1).

Table 1: Changes in myocardial enzymes (per group)

	LDH (U/L)	CK (U/L)	CK-MB (U/L)
Control	369.32±32.365	149.28±23.349	521.86±35.323
DOX	957.94±40.94#	489.38±26.426#	1029.08±62.134#
ESC(low)+DOX	853.04±51.206**	421.3±48.047*	819.64±32.187***
ESC(high)+DOX	495.16±32.917***	201.28±20.366***	583.24±46.112***

\*  $p < 0.05$  versus DOX group, \*\*  $p < 0.01$  versus DOX group, \*\*\*  $p < 0.001$  versus DOX group, #  $p < 0.05$  versus control group.

2. Effect of ESC on myocardial structure of rats after DOX intervention

After Masson staining, the muscle fibers turn red and the collagen fibers turn blue, which can directly show the collagen deposition in the myocardial tissue. The myocardial fibers in the control group were red, orderly arranged in a strip shape, and few blue collagen fibers (Figure 1a). In DOX group, the cells of rats were

vacuolar denatured, a large number of collagen fibers were dyed blue, interconnected into a network, disorderly arranged and unevenly distributed (Figure 1b). Compared with DOX group, the blue collagen fibers in ESC low dose+DOX group decreased slightly (Figure 1c). Blue collagen fibers decreased significantly in ESC high dose+DOX group.

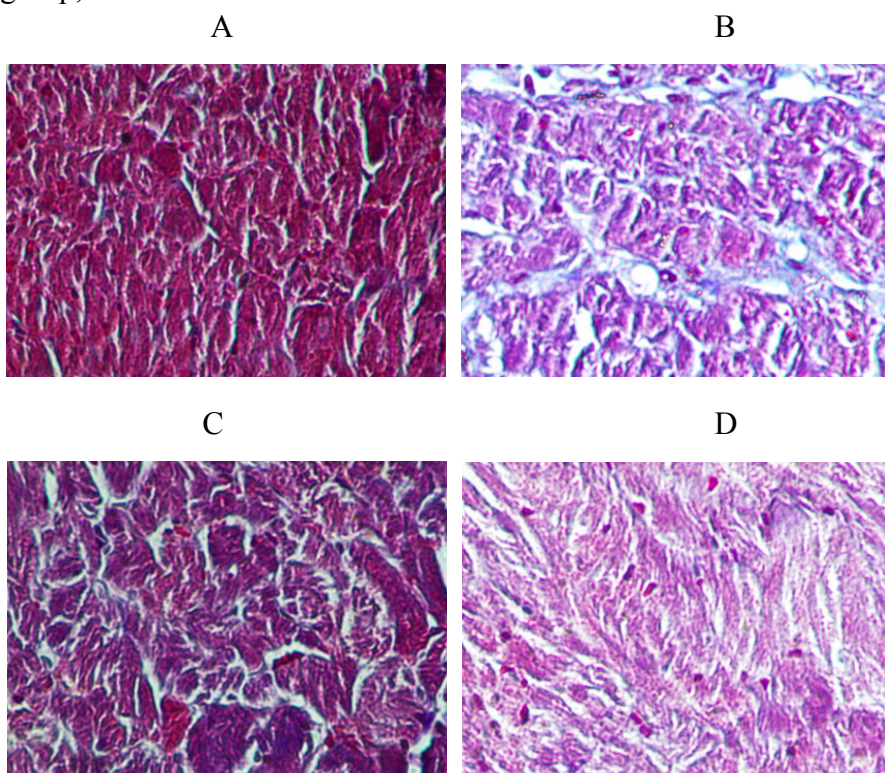


Figure 1: The changes of collagen fibers in myocardial tissue of rats in each group were observed under Masson staining (X200).

**Discussion**

Anthracycline antitumor antibiotics are an important class of chemotherapeutic drugs, but long-term application can lead to dose-dependent irreversible cardiomyopathy, causing serious cardiotoxicity and liver damage. The toxicity of doxorubicin mainly comes from the destruction of mitochondrial function and the

production of damaging oxygen free radicals.<sup>4</sup> Due to the lack of antioxidant enzymes, cardiomyocytes have a low level of antioxidant resistance and stronger sensitivity to free radicals, which makes the heart injury more serious. Therefore, inhibiting the production of reactive oxygen species may reduce the cardiotoxicity of doxorubicin. Lactate

dehydrogenase, creatine kinase and creatine kinase isoenzyme MB are valuable in the diagnosis of myocardial injury, craniocerebral injury and liver diseases. It is generally believed that CK and CK-MB can specifically reflect the injury of cardiomyocytes.<sup>5</sup> The degree of cell damage caused by doxorubicin can be judged by measuring serum myocardial enzymes. This study showed that serum LDH, CK and CK-MB increased significantly after doxorubicin injury. After ESC intervention, myocardial enzyme indexes decreased in varying degrees, especially in the high-dose group. It showed that ESC had a certain protective effect on doxorubicin induced myocardial injury in a dose-dependent manner. Collagen is the structural part of human organs and tissues, which is closely related to the body function. Under pathological conditions, collagen will change and affect the function of tissues and organs.<sup>6</sup> When there is too much collagen in the heart, it will affect the systolic, diastolic function, cardiac output, causing a series of changes in neurophysique, and leading to ventricular remodeling.<sup>7</sup> The main pathological feature of doxorubicin induced myocardial injury model in this experiment was the increase of myocardial interstitial collagen fiber content. The degree of myocardial tissue injury in rats after ESC intervention was significantly changed compared with DOX group. This study showed that ESC had a correlation with doxorubicin induced

myocardial histopathology.

At present, the mechanism of doxorubicin induced myocardial injury is not clear, but inhibiting the production of oxygen free radicals or directly eliminating excess oxygen free radicals can help to reduce the cardiotoxicity of doxorubicin. Although there is no recognized effective oral drug against adriamycin cardiotoxicity, which makes no positive control group in this experiment, the results of this study have provided a theoretical and experimental basis for further research.

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