


# Yangxin granules alleviate doxorubicin-induced cardiotoxicity by suppressing oxidative stress and apoptosis mediated by AKT/GSK3 $\beta$ / $\beta$ -catenin signaling

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## Abstract

**Background:** Yangxin granules (YXC), a Chinese herbal medicine, have been confirmed to have clinical benefits in the treatment of heart failure. This study examined the effects and molecular mechanisms of YXC in the treatment of doxorubicin-induced cardiotoxicity *in vitro*.

**Methods:** H9c2 cardiomyocytes were pretreated with YXC (5, 10, or 20 mg/mL) or the AKT inhibitor MK-2206 (50 nM) before doxorubicin treatment (1  $\mu$ M). Cell apoptosis, viability, inflammatory factor expression (TNF- $\alpha$ , IL-1 $\beta$ , and IL-6), and oxidative stress mediator levels including superoxide dismutase, reactive oxygen species, and malondialdehyde were detected.

**Results:** YXC increased the viability of H9c2 cells. In addition, doxorubicin inhibited AKT/GSK3 $\beta$ / $\beta$ -catenin signaling, whereas YXC increased the expression of phosphorylated AKT and GSK3 $\beta$ , and  $\beta$ -catenin in doxorubicin-treated H9c2 cells. Moreover, T-cell factor/lymphoid enhancer factor signaling downstream of  $\beta$ -catenin was also activated by YXC. YXC pretreatment also inhibited doxorubicin-induced inflammation, oxidative stress, and apoptosis. However, MK-2206 reversed the effects of YXC in doxorubicin-treated H9c2 cells.

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**Conclusions:** YXC alleviates doxorubicin-induced inflammation, oxidative stress, and apoptosis in H9c2 cells. These effects might be mediated by the AKT/GSK3 $\beta$ / $\beta$ -catenin signaling pathway. YXC might have preventive effects against doxorubicin-induced heart failure.

## Keywords

Heart failure, apoptosis, oxidative stress, doxorubicin, Yangxin granules, AKT, GSK3 $\beta$ ,  $\beta$ -catenin

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## Introduction

Heart failure, which emerges when the heart is damaged or weakened, occurs when cardiac muscle cannot pump blood efficiently. Certain conditions, such as high blood pressure, coronary artery disease, diabetes, and the use of certain medications, can lead to heart failure.<sup>1</sup> Chemotherapeutics such as paclitaxel, doxorubicin, and cisplatin have essential roles in cancer treatment. Doxorubicin is a pervasive first-line treatment for lymphomas. However, it was revealed in 1979 that doxorubicin induces congestive heart failure.<sup>2</sup> Therefore, protection of the heart during doxorubicin treatment is of great importance. Several strategies have been developed to prevent doxorubicin-induced heart damage, such as the use of low drug doses, liposomal encapsulation of the drug, or combination use with cardioprotective drugs such as dexrazoxane. However, additional modalities are needed to protect the heart against the damaging effects of doxorubicin. The use of doxorubicin is also severely limited by its dose-dependent toxicities. Doxorubicin binds to nitric oxide synthase to promote the generation of superoxide and subsequent increases in the generation of free radicals, which can induce oxidative stress.<sup>3</sup> In addition, doxorubicin can induce apoptosis by stimulating cytochrome C release from mitochondria.<sup>4</sup> This acceleration of oxidative

stress is a potential damaging factor for cardiomyocytes.

Traditional Chinese medicines (TCMs) have been used to cure various diseases in China and other Asian countries for thousands of years. Herbal medicines and other formulations have proven effective and reliable for some diseases. For example, Qishen Yiqi dropping pills ameliorate cardiac remodeling by inhibiting the left anterior descending ligation induced-upregulation of collagen I, collagen III, and matrix metalloproteinase 2.<sup>5</sup> Berberine prevents heart ischemia/reperfusion injury by regulating endoplasmic reticulum stress.<sup>6</sup> Yangxin granules (YXC) consist of Zhifupian (*Aconitum carmichaelii* Debx.), Guizhi (*Radix glycyrrhizae*), Fuling (*Rhizoma smilacis glabrae*), Zexie (*Rhizoma Alismatis*), Huangjing (*Rhizoma Polygonati*), Maidong (*Radix Ophiopogonis japonici*), Chuanniuxi (*Radix Cyathulae officinalis*), and Dansen (*Radix Salvia Miltiorrhiza*). The treatment was developed by Shaanxi Traditional Chinese Medicine Hospital based on clinical experience. The therapy mainly has effects on “heart-kidney yang deficiency” and “blood stasis with water retention,” which are primarily manifested as palpitation and edema in heart failure. Therefore, in the current investigation, we investigated whether YXC could prevent doxorubicin-induced cardiotoxicity in an *in vitro* model and further explore its latent mechanisms.

## Materials and Methods

### Reagents

Dulbecco's modified eagle medium (DMEM) powder (Gibco) and fetal bovine serum (FBS, Gibco) was purchased from Thermo Fisher Scientific (Waltham, MA, USA).  $\text{NaHCO}_3$ , the TOX1 MTT-based *in vitro* toxicology assay kit, and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich (St. Louis, MO, USA). YXC was obtained from Shaanxi Traditional Chinese Medicine Hospital (Xi'an, China). To create the stock solution, 100 mg of YXC powder were dissolved in 1 mL of PBS.

### Cell culture

H9c2 rat cardiomyocytes were obtained from the Shanghai Institutes for Biological Sciences (Shanghai, China). DMEM powder was dissolved in PBS supplemented with  $\text{NaHCO}_3$  (1.5 g/L). Cells were cultured in complete culture medium containing DMEM supplemented with 10% FBS in a 37°C incubator.

### MTT

H9c2 cells ( $2 \times 10^3$  cells/well) were seeded into a 96-well plate. After attachment to the plate bottom, cells were treated with 1, 5, 10, or 20 mg/mL YXC at for 8, 12, or 24 hours. H9c2 cells ( $3.5 \times 10^3$  cells/well) were separately seeded in a 96-well plate and pre-treated with YXC (5, 10, or 20 mg/mL) or co-treated with YXC (20 mg/mL) and the AKT inhibitor MK-2206 (50 nM) for 4 hours. Then, doxorubicin (1  $\mu\text{M}$ ) was added for co-treatment with YXC for another 24 hours. MTT solution (20  $\mu\text{L}$ /well) was added to the plate, which was then incubated at 37°C for 4 hours. Then, the culture medium was removed, and 150  $\mu\text{L}$  of DMSO were added to each well. The absorbance value at 570 nm was measured using

a Varioskan LUX multimode reader (Thermo Fisher Scientific).

### Western blotting

H9c2 cells ( $5 \times 10^5$  cells/well) were seeded into a six-well plate. Then, cells were pre-treated with YXC (5, 10, or 20 mg/mL) or co-treated with YXC (20 mg/mL) and MK-2206 (50 nM) for 4 hours. Doxorubicin (1  $\mu\text{M}$ ) was added for co-treatment with YXC for another 24 hours. Total proteins were extracted from cells using RIPA lysis and extraction buffer (Thermo Fisher Scientific), and nuclear proteins were isolated using a Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime Biotechnology, Shanghai, China) according to the manufacturer's instructions. Next, 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis was performed to fractionate the protein samples. The subsequent protocols were performed as previously reported.<sup>7</sup> Primary antibodies were used against p-AKT, AKT, p-GSK3 $\beta$ , GSK3 $\beta$ ,  $\beta$ -catenin, Bax, Bcl-2, cleaved caspase-3, caspase-3, p65, lamin B1, and GAPDH (all from Abcam, Cambridge, UK).

### TOP/FOP Flash reporter assay

This assay was performed to determine the transcriptional activity of  $\beta$ -catenin in cells. The T-cell factor (TCF) reporter plasmid kit was purchased from Sigma-Aldrich. Briefly, H9c2 cells ( $5 \times 10^4$  cells/well) were seeded into 24-well plates and transfected with TOP Flash or FOP Flash plasmid before treatment. Cells were treated with doxorubicin and YXC as described for western blotting. Luciferase activity was determined using the Dual-Luciferase Reporter Assay (Promega, Madison, WI, USA) 24 hours after cell treatment. *Renilla* luciferase activity was examined for normalization in each sample.

### **Inflammation detection**

Rat TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 ELISA kits were purchased from Abcam (Cambridge, UK). To investigate the secretion of inflammatory factors, H9c2 cells ( $5 \times 10^5$  cells/well) were seeded into a six-well plate. Then, cells were pre-treated with YXC (5, 10, or 20 mg/mL) or co-treated with YXC (20 mg/mL) and MK-2206 (50 nM) for 4 hours. Then, cells were treated with doxorubicin (1  $\mu$ M) for 24 hours to induce inflammation. The supernatant of treated cells was collected, and the concentrations of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 were detected according to the instructions of the commercial assay kits as described previously.<sup>8</sup>

### **Oxidative stress assay**

H9c2 cells ( $2.5 \times 10^3$  cells/well) were seeded into a 96-well plate. After overnight attachment, cells were stained with DCFDA for 45 min and then examined using a Cellular Reactive Oxygen Species (ROS) Detection Assay Kit (Abcam) following the manufacturer's instructions. H9c2 cells ( $2.5 \times 10^3$  cells/well) were seeded into a 96-well plate. After attachment, cells were lysed using lysis buffer to obtain the protein sample. Then, samples were detected using a Human/Mouse/Rat Total superoxide dismutase (SOD) 2/Mn-SOD DuoSet IC ELISA kit (R&D Systems, Minneapolis, MN, USA). H9c2 cells ( $1 \times 10^4$  cells/well) were seeded into a 24-well plate. After treatment, the supernatant was collected to determine the levels of lactate dehydrogenase (LDH) and malondialdehyde (MDA) using relevant assay kits (Abcam).

### **Flow cytometry for apoptosis**

Cell apoptosis was evaluated via flow cytometry using an Annexin V-FITC/PI Apoptosis detection kit (Vazyme, Nanjing, China). In brief, treated H9c2 cells were digested with 0.25% trypsin without

EDTA for 5 minutes and resuspended in binding buffer. Cells were subsequently stained with 5  $\mu$ L of Annexin V-FITC and 5  $\mu$ L of PI for 10 min in the dark. Then, 400  $\mu$ L of binding buffer were added followed by analysis using a CytoFLEX flow cytometer (Beckman Coulter, Brea, CA, USA).

### **Statistical analysis**

GraphPad Prism 6.0 (GraphPad, San Diego, CA, USA) was used to analyze the differences between groups. The significance was evaluated using one-way ANOVA followed by Tukey's test. Significance was defined as  $P < 0.05$ .

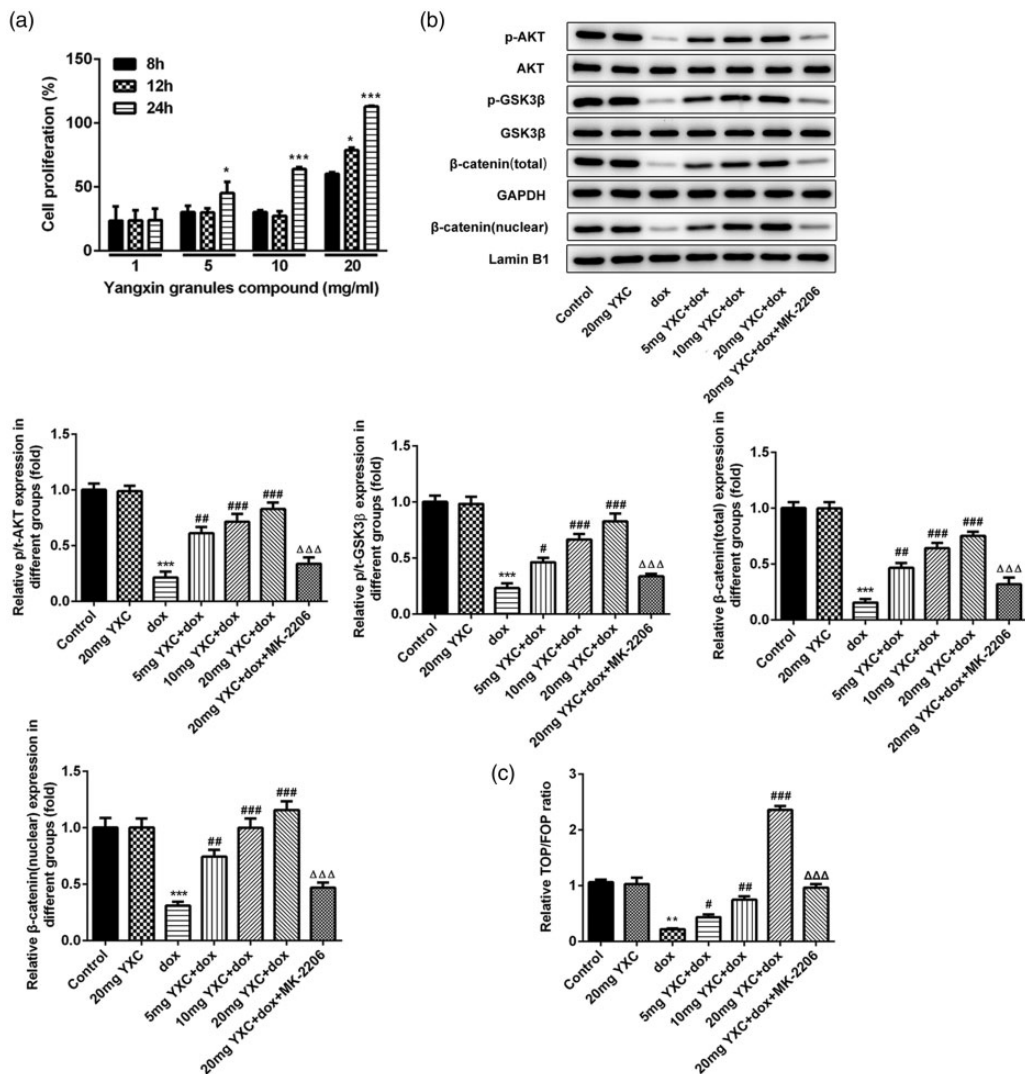
### **Ethics and consent statement**

The study did not include human or animal investigations, and thus, ethics approval and informed consent were not required.

## **Results**

### ***YXC promotes the proliferation of H9c2 cells and increases AKT/GSK3 $\beta$ / $\beta$ -catenin signaling***

To evaluate the effects of YXC on cardiomyocytes, we initially examined the viability of H9c2 rat cardiomyocytes. The results indicated that YXC treatment for 24 hours dramatically promoted the proliferation of H9c2 cells ( $P < 0.05$ , Figure 1a). According to previous research,<sup>9</sup> H9c2 cells treated with 1  $\mu$ M doxorubicin exhibited signs of cardiotoxicity. Furthermore, western blotting revealed that YXC dramatically increased the expression of p-AKT, p-GSK3 $\beta$ , and total and nuclear  $\beta$ -catenin in doxorubicin-treated H9c2 cells (Figure 1b). To confirm the effects of YXC on AKT/GSK3 $\beta$ / $\beta$ -catenin signaling, TOP/FOP Flash analysis was performed to assess signaling downstream of  $\beta$ -catenin, namely the TCF/lymphoid enhancer factor

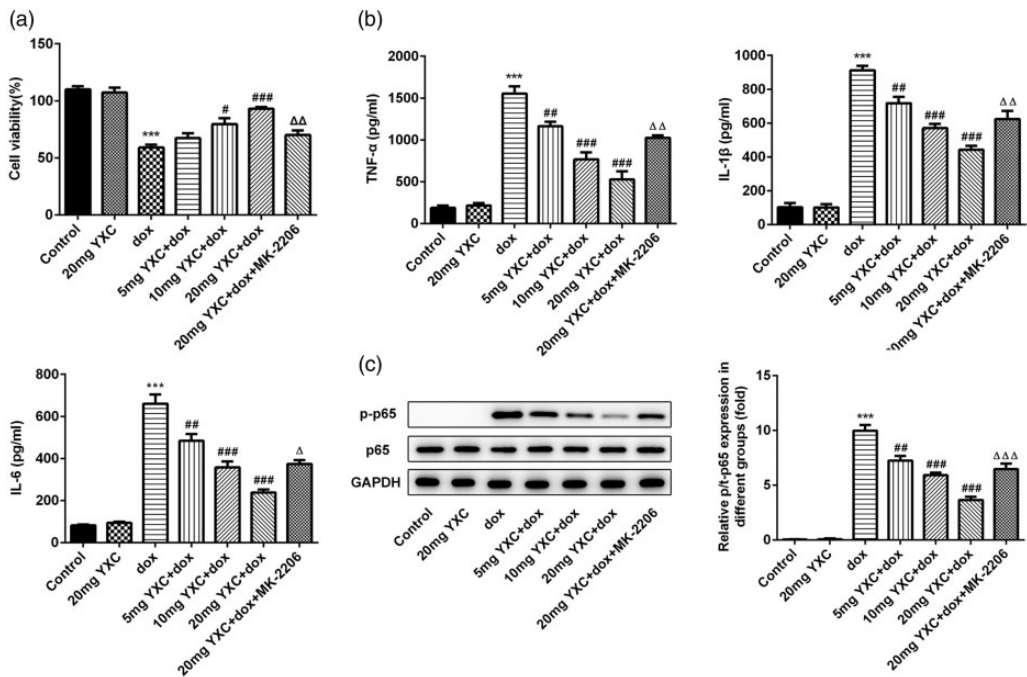


**Figure 1.** Yangxin granules (YXC) promoted H9c2 cell proliferation and AKT/GSK3 $\beta$ / $\beta$ -catenin pathway. (a) The MTT assay was performed to detect the viability of H9c2 cells following treatment with YXC (1, 5, 10, or 20 mg/ml) for 8, 12, or 24 hours. \* $P < 0.05$ , \*\*\* $P < 0.001$  versus 8 hours. (b) p-AKT, p-GSK3 $\beta$ , and total and nuclear  $\beta$ -catenin expression after treatment with YXC or an AKT inhibitor (MK-2206) was detected using western blotting. (c) The transcriptional activity of the downstream  $\beta$ -catenin pathway T-cell factor/lymphoid enhancer factor (TCF/LEF) was detected using the TOP/FOP Flash reporter assay. YXC+dox, treatment with YXC (5, 10, or 20 mg/ml) and subsequent co-treatment with 1  $\mu$ M doxorubicin. 20 mg+dox+MK-2206, treatment with YXC (20 mg/ml) and MK-2206 (50 nM) and subsequent co-treatment with 1  $\mu$ M doxorubicin. \*\* $P < 0.01$ , \*\*\* $P < 0.001$  versus control. # $P < 0.05$ , ## $P < 0.01$ , ### $P < 0.001$  versus doxorubicin.  $\Delta\Delta\Delta P < 0.001$  versus 20 mg YXC+dox.

(LEF) transcription family. As presented in Figure 1c, H9c2 cells treated with 1  $\mu$ M doxorubicin exhibited a smaller TOP/FOP ratio ( $P < 0.01$ ), indicating that doxorubicin reduced  $\beta$ -catenin-dependent TCF transcriptional activity, whereas the addition of YXC reversed these effects. MK-2206 similarly repressed the activation of AKT/GSK3 $\beta$ / $\beta$ -catenin signaling induced by YXC (Figure 1b) and further reduced  $\beta$ -catenin/TCF transcriptional activity ( $P < 0.05$ , Figure 1c). These data manifested that YXC promoted the viability of H9c2 cells and enhanced AKT/GSK3 $\beta$ / $\beta$ -catenin signaling in doxorubicin-treated H9c2 cells.

### Increased viability and decreased inflammation in doxorubicin-induced H9c2 cells

Next, we detected the viability of doxorubicin-treated H9c2 cells using the MTT assay. After treatment with YXC for 24 hours, YXC drastically reversed the decrease of cell viability induced by doxorubicin ( $P < 0.05$ ), whereas MK-2206 inhibited the effects of YXC ( $P < 0.01$ , Figure 2a). In addition, the doxorubicin-induced secretion of inflammatory factors, namely TNF- $\alpha$ , IL-1 $\beta$ , and IL-6, was remarkably suppressed by YXC (all  $P < 0.05$ , Figure 2b). However, MK-2206



**Figure 2.** Yangxin granule (YXC) treatment suppressed doxorubicin-induced cytotoxicity and inflammation in H9c2 cells. (a) Cell viability was detected using the MTT assay. (b) Levels of the pro-inflammatory factors TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 were detected using ELISA. (c) Phosphorylation of p65 after treatment with YXC or the AKT inhibitor MK-2206 was detected using western blotting. YXC+dox, treatment with YXC (5, 10, or 20 mg/mL) and subsequent co-treatment with 1  $\mu$ M doxorubicin. 20 mg+dox+MK-2206, treatment with YXC (20 mg/mL) and MK-2206 (50 nM) and subsequent co-treatment with 1  $\mu$ M doxorubicin. \*\*\* $P < 0.001$  versus control. # $P < 0.05$ , ### $P < 0.01$ , #### $P < 0.001$  versus dox. ΔΔ $P < 0.01$ , ΔΔΔ $P < 0.001$  versus 20 mg YXC+dox.

reversed the pro-inflammatory effects of YXC (all  $P < 0.05$ , Figure 2b). At the same time, the activation of NF- $\kappa$ B induced by doxorubicin was inhibited by co-treatment with YXC, which was demonstrated by the expression of p-p65 (Figure 2c).

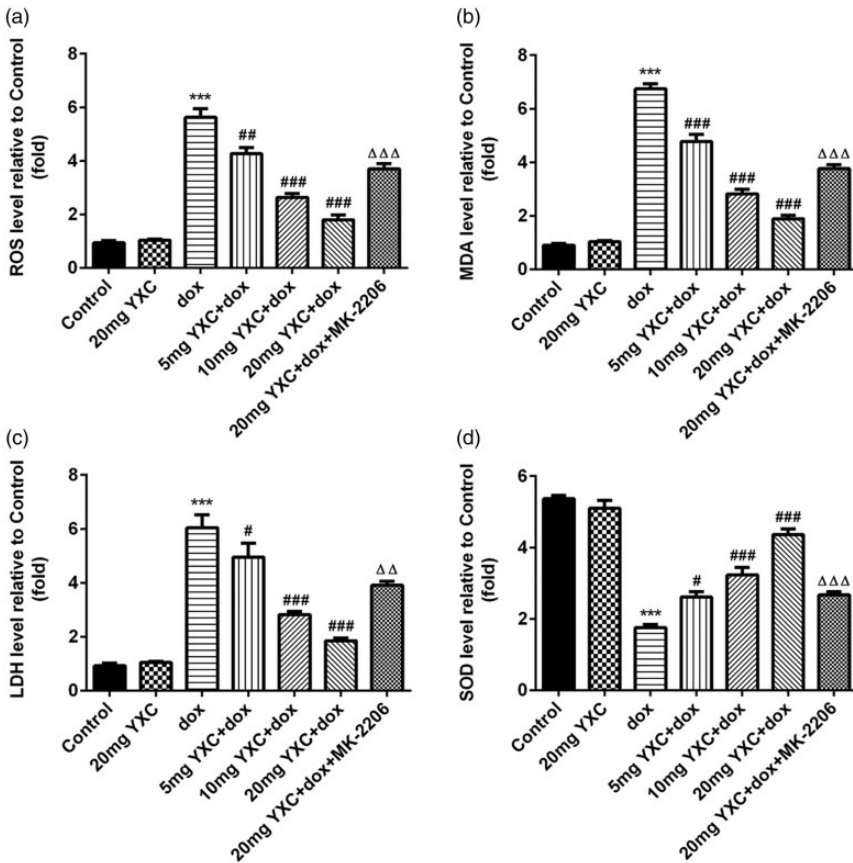
### Inhibition of oxidative stress by YXC in doxorubicin-induced H9c2 cells

Compared with the findings in control cells, ROS and MDA generation was substantially increased in doxorubicin-treated H9c2

cells (both  $P < 0.001$ , Figure 3a and 3b). In addition, the protective effect of YXC was verified by the increase of SOD activity ( $P < 0.05$ , Figure 3d). Such results defined a beneficial role of YXC in doxorubicin-induced oxidative injury.

### Inhibition of apoptosis by YXC in doxorubicin-treated H9c2 cells

Furthermore, oxidative stress is usually accompanied by cell death. Doxorubicin-induced LDH expression, which indicated

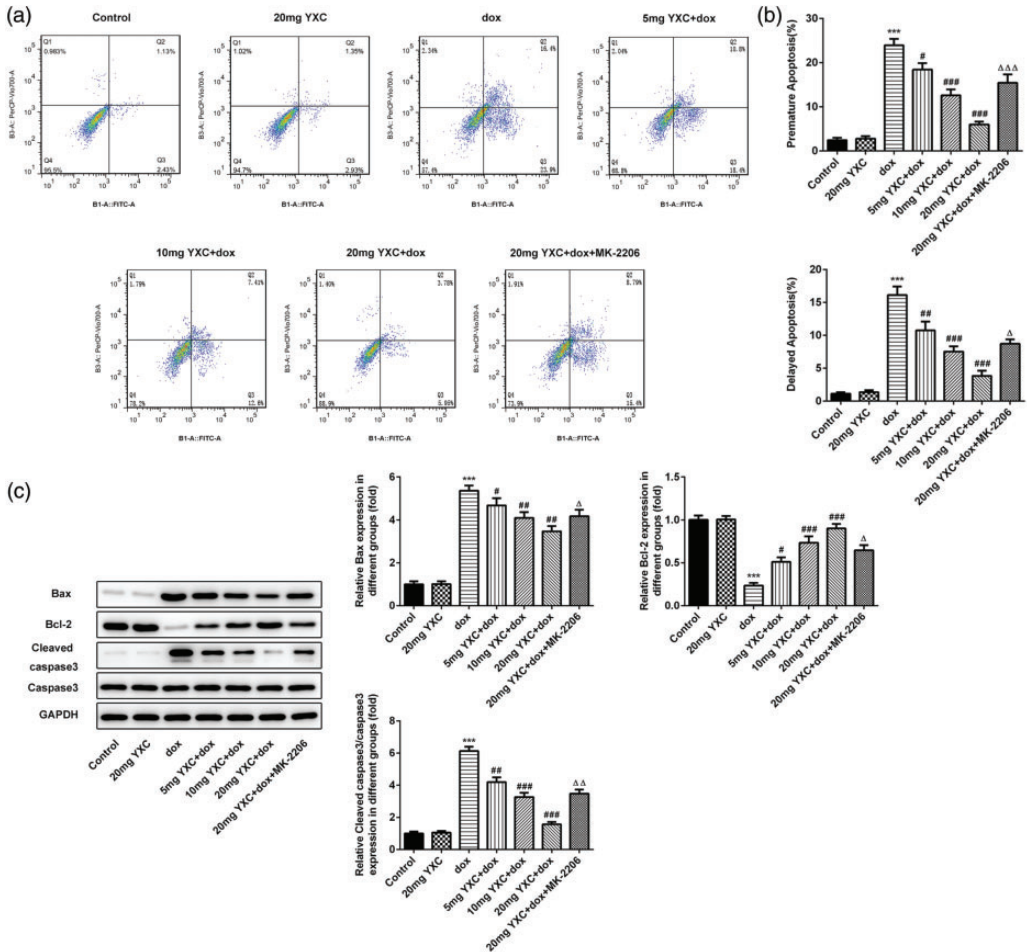


**Figure 3.** Yangxin granules (YXC) reduced doxorubicin-induced oxidative stress and cytotoxicity in H9c2 cells. (a) Reactive oxygen species (ROS), (b) malondialdehyde (MDA), (c) lactate dehydrogenase (LDH), and (d) superoxide dismutase (SOD) were detected using a commercial kit. YXC+dox, treatment with YXC (5, 10, or 20 mg/mL) and subsequent co-treatment with 1  $\mu$ M doxorubicin. 20 mg+dox+MK-2206, treatment with YXC (20 mg/mL) and MK-2206 (50 nM) and subsequent co-treatment with 1  $\mu$ M doxorubicin.

\*\*\* $P < 0.001$  versus control. ### $P < 0.01$ , #### $P < 0.001$  versus dox.  $\Delta\Delta\Delta P < 0.001$  versus 20 mg YXC+dox.

cell death, was prominently suppressed by YXC ( $P < 0.05$ , Figure 3c). Conversely, MK-2206 dramatically reversed the down-regulation of LDH by YXC in doxorubicin-treated H9c2 cells ( $P < 0.01$ , Figure 3c). Cardiomyocyte apoptosis is a crucial component of the doxorubicin-induced

cytotoxicity. Our results illustrated that YXC alleviated doxorubicin-induced apoptosis ( $P < 0.05$ , Figure 4a–4b). The reduction of apoptosis was further demonstrated using western blotting, which revealed that YXC reversed the downregulation of Bax and cleaved caspase-3 and upregulation of



**Figure 4.** Yangxin granules compound (YXC) reduced doxorubicin-induced apoptosis in H9c2 cells. (a and b) Flow cytometry analysis was performed to detect apoptotic cells. (c) The levels of apoptotic proteins including Bax, Bcl-2, cleaved caspase-3, and caspase 3 were assessed using western blotting. YXC+dox, treatment with YXC (5, 10, or 20 mg/mL) and subsequent co-treatment with 1  $\mu$ M doxorubicin. 20 mg+dox+MK-2206, treatment with YXC (20 mg/mL) and MK-2206 (50 nM) and subsequent co-treatment with 1  $\mu$ M doxorubicin. \*\*\* $P < 0.001$  versus control. # $P < 0.05$ , ### $P < 0.01$ , #### $P < 0.001$  versus dox.  $\Delta P < 0.05$ ,  $\Delta\Delta P < 0.01$ ,  $\Delta\Delta\Delta P < 0.001$  versus 20 mg YXC+dox.



Bcl-2 induced by doxorubicin (all  $P < 0.05$ ); however, MK-2206 reversed the effects of YXC (all  $P < 0.05$ , Figure 4c). Thus, YXC inhibited doxorubicin-induced apoptosis, possibly by upregulating AKT/GSK3 $\beta$ / $\beta$ -catenin signaling.

## Discussion

Doxorubicin is an effective anti-cancer drug; however, its use is limited by its cardiac side effects. Our previous 35-patient study illustrated that YXC is an effective treatment for chronic heart failure (CHF).<sup>10</sup> The results demonstrated that doxorubicin induced cardiotoxicity by increasing the levels of inflammatory factors including TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 and inducing cardiomyocyte oxidative stress and apoptosis in H9c2 cells, in line with previous investigations.<sup>11,12</sup> As components of YXC, *Ophiopogon japonicus* and *Radix Salvia Miltiorrhiza* are herbs that have protective effects on the heart. A previous investigation indicated that polysaccharide from *Ophiopogon japonicus* exerts protective effects against myocardial ischemia injury.<sup>13</sup> Diethyl blechnic, salvianolic acid B, cryptotanshinone, and tanshinone IIA extracted from *Salvia Miltiorrhiza* Bunge have been proven to prevent doxorubicin-induced cardiotoxicity both *in vitro* and *in vivo*.<sup>14-17</sup> The protective effects of YXC on doxorubicin-induced cardiotoxicity were initially and preliminarily demonstrated in the present *in vitro* study. However, the detailed effective constituent and mechanism require additional research.

H9c2 cells displayed dramatically increased proliferation after 24 hours of treatment with YXC, indicating the potential protective effects of YXC against cardiomyocyte injury. Subsequently, YXC was used to treat doxorubicin-treated H9c2 cells. Chronic pro-inflammatory factors, such as TNF- $\alpha$ , IL-1 $\beta$ , and IL-6, contribute to the pathogenesis of CHF by inducing

myocardial remodeling, endothelial dysfunction, and vascular injury.<sup>18</sup> In addition, TNF- $\alpha$  levels in circulation are potential predictors in patients with heart failure, and the inhibition of TNF- $\alpha$  exerts protective roles in animal experiments.<sup>19,20</sup> YXC significantly inhibited the doxorubicin-induced secretion of the pro-inflammatory factors TNF- $\alpha$ , IL-1 $\beta$ , and IL-6. The activation of NF- $\kappa$ B signaling can induce target gene transcription and generate cellular responses, such as immunity, inflammation, and stress. NF- $\kappa$ B is a protein complex consisting of p65 and p50.<sup>21</sup> Our results indicated that doxorubicin enhanced p-p65 expression, indicating the activation of NF- $\kappa$ B signaling. However, YXC co-treatment remarkably blunted the expression of p-p65. These results indicated that YXC reduced doxorubicin-induced heart failure by regulating inflammation. Furthermore, oxidative stress triggers mitochondrial dysfunction and myocardial necroptosis in heart failure.<sup>22</sup> YXC also repressed oxidative stress, as manifested by reductions ROS and MDA generation and an increase of SOD generation in doxorubicin-treated H9c2 cells. YXC reduced doxorubicin-induced apoptosis in H9c2 cells and the upregulation of apoptotic proteins including Bax, Bcl-2, and cleaved caspase-3, which are narrowly associated with mitochondrial function.<sup>23</sup> Therefore, YXC could inhibit doxorubicin-induced inflammation, oxidative stress, and apoptosis and subsequently prevent heart failure.

Recent investigations found that various components from TCMs activate the AKT/GSK3 $\beta$ / $\beta$ -catenin signaling pathway. Salvianolic acid B stimulates the PI3K/AKT/GSK3 $\beta$ / $\beta$ -catenin pathway to induce neural differentiation.<sup>24</sup> Bufalin inhibits the metastasis of hepatocellular carcinoma by inhibiting the AKT/GSK3 $\beta$ / $\beta$ -catenin/E-cadherin signaling pathway.<sup>25</sup> Huaier aqueous extract inhibits PI3K/AKT/

GSK3 $\beta$ / $\beta$ -catenin signaling, thereby exerting anti-cancer effects.<sup>26</sup> PI3K/AKT pathway blockade inhibits the Wnt/ $\beta$ -catenin pathway mediated by GSK3 $\beta$  activation and further suppresses cardiomyocyte differentiation.<sup>27</sup> Cardiomyocyte differentiation regenerates and restores cardiac function and reduces myocardial injury. Ang-II can induce AKT/GSK3 $\beta$ / $\beta$ -catenin/TCF/LEF signaling and suppress myocardial hypertrophy in rats.<sup>28</sup> AKT/GSK3 $\beta$ / $\beta$ -catenin phosphorylation alleviates inflammation, thereby blocking hypertrophic effects.<sup>29</sup> The current study results revealed that YXC dramatically increased the expression of p-AKT, which can phosphorylate and thus inactivate GSK3 $\beta$  and subsequently stabilize  $\beta$ -catenin. At the same time, doxorubicin inhibited the secretion of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 in H9c2 cells, whereas MK-2206 reversed the effects of YXC. In addition, MK-2206 reversed the suppressive effects of YXC on oxidative stress and apoptosis. Importantly, we found that YXC treatment alone did not affect inflammation, apoptosis, or oxidative stress in H9c2 cells. Therefore, YXC might protect against doxorubicin-induced inflammation, oxidative stress, and apoptosis by increasing AKT/GSK3 $\beta$ / $\beta$ -catenin signaling without any side effects.

In conclusion, the current investigation identified a potential therapeutic role for YXC in reducing cardiotoxicity during doxorubicin treatment. However, further *in vivo* and clinical investigations of YXC in the treatment of doxorubicin-induced cardiotoxicity are needed.

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Not applicable.

### Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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