

**Danshen (*Salvia miltiorhiza*) mediates estrogen receptor activate Akt inhibition of
Leu27IGF-II-induced IGF-II receptor signaling activation in cardiomyoblaste
apoptosis**

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Abstract

Aim of the study: Danshen (*Salvia miltiorrhiza*), are widely used in China for the treatment of cardiovascular disorders, including coronary heart disease. Tanshinone IIA, a lipid-soluble biologically active component isolated from Danshen, this compound chemical structure similar to 17 β -estradiol (E2). Therefore, pretreatment of cardiomyoblast cells with ICI 182,780 (ICI), an estrogen receptor antagonist to investigated the estrogenic activity of Danshen. To further identify the cardioprotective effect and molecular mechanisms of Danshen and ICI, we investigated the effects of Danshen extract and ERs antagonist on Leu27IGF-II pathway-induced apoptosis by analyzing the activation of survival and G α q signaling pathways in H9c2 cardiomyoblast cells.

Materials and methods: H9c2 cells induced apoptosis with Leu27IGFII (10^{-8} M) administration. H9c2 cells were divided into five groups: Control, Leu27IGF-II (10^{-8} M), two Danshen extracts (0.5 and 1 μ g/mL) plus Leu27IGF-II and ICI plus Danshen extracts (1 μ g/mL) with L27IGF-II. We detected apoptosis using TUNEL assay. JC-1 staining and Western blot were used to detect IGF-IIR signaling proteins, pro-apoptotic proteins and survival pathway (p-Akt, Bcl-xL and p-Bad).

Results: We found that treatments Danshen extracts significantly phosphorylated Akt that mediated through estrogen receptor to inhibited Leu27IGF-II-induced apoptosis analyzed by TUNEL assay, and Western blot for IGF-IIR signaling proteins (including IGF-IIR, G α q and calcineurin) and mitochondrial death signaling proteins, released cytochrome c and active caspase-3. However, the cardioprotective properties of Danshen to inhibit Leu27IGF-II-induced apoptosis and promote cell survival were attenuated by applying ICI. The findings suggest that cardio protective effect of Danshen is mediated through estrogen receptors.

Conclusions: All data suggest that Danshen exerts estrogenic activity against IGF2R signaling induced cardiac apoptosis, and acts as a potential cardioprotective TCM.