The Protective Role of Glutathione on Doxorubicin-Induced Cardiotoxicity in Human Cardiac Progenitor Cells

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Abstract

Cardiotoxicity caused by doxorubicin (DOX) is an important issue to consider for both patients and doctors who require DOX. DOX-induced cardiotoxicity is closely associated with cardiomyocyte death and dysfunction. To prevent DOX-induced cardiotoxicity, many studies have been conducted on new therapeutic strategies, including the discovery of novel functional modulators such as antioxidant drugs to restore the loss of function of transplanted or residual cardiac cells in the heart. We investigated whether glutathione (GSH), an antioxidant drug, has a protective effect against DOX-induced cardiotoxicity by decreasing ROS and unraveling the underlying molecular mechanisms. GSH clearly increased the viability of damaged human cardiac progenitor cells (hCPCs) treated with DOX. In addition, ROS generation and apoptosis induced by DOX treatment were significantly reduced. We also observed that GSH restored the capacity of hCPCs, as shown by the wound healing assay, transwell migration, and tube formation. We checked that GSH treatment restored the level of pERK, which increased in the DOX-treated group. The ERK inhibitor, U0126, increased the viability of damaged hCPCs. These data suggest that the restoration mechanism of GSH may be via the regulation of pERK signaling. We confirmed the effects of DOX and GSH using an in vivo model. As a result, GSH was confirmed to have a protective effect against DOX-induced cardiotoxicity through body weight, survival rate, histology, and mRNA level. Taken together, GSH prevents DOX-induced cardiotoxicity and regulates pERK signaling. GSH may be an effective therapeutic strategy for DOX-induced cardiotoxicity.

Introduction

Doxorubicin (DOX) is an anticancer agent belonging to the anthracycline class of drugs that is used to treat breast cancer, bladder cancer, Kaposi's sarcoma, lymphoma, and acute lymphocytic leukemia [1, 2]. However, its application is associated with side effects, such as myocardial dysfunction, dilated cardiomyopathy, and heart failure [3, 4]. The mechanism of DOX-mediated side effects is multifactorial, including intercalation of DNA, ROS generation, and mitochondrial dysfunction [5, 6]. Among these, we focused on ROS inhibition, which has been the most studied.

The adult heart is well known as an organ where differentiation is completed. Since the beginning of the 2000s, research on stem cells present in the heart has been intensively studied [7–10]. Cardiac progenitor cells (CPCs) were discovered in adult mouse hearts by Anversa et al. in 2003 [11]. The human CPCs (hCPCs) are self-renewing, clonogenic, and multipotent in that they can differentiate into cardiomyocytes, smooth muscle cells, and endothelial cells [12]. In addition, they have been reported to proliferate and differentiate into damaged cardiomyocytes and contribute to regeneration [11, 13, 14]. Therefore, cardiomyocyte recovery is important, achieved by protecting or restoring hCPCs in the damaged heart.

Glutathione (GSH) is a well-known antioxidant composed of amino acids including glutamic acid, cysteine, and glycine, and its reduced form protects cells by reducing ROS. Previous studies have shown that GSH protects against DOX-induced myocardial toxicity [15, 16], and a recent study tested this result
using animal experiments [17]. However, information regarding GSH and DOX is limited and the underlying molecular mechanisms are unknown.

Previous studies have reported that DOX-induced cardiotoxicity leads to ROS generation, including peroxide, superoxide, and hydroxyl radicals, and blocking of calcium channels by antioxidants [18, 19]. DOX-damaged cardiomyocytes, such as AC16 and H9C2, have been reported to be associated with diverse molecular mechanisms involving the Nrf2, AKT, and ERK pathways [20–23]. In addition, research on cancer cells and DOX has progressed steadily [24, 25]. The alleviation of DOX-induced cardiomyopathies using hCPCs has also been studied [26]. However, the mechanism underlying the treatment with antioxidants in hCPCs damaged by DOX is unclear.

In this study, we hypothesized that GSH, an antioxidant, relieves DOX-induced damage caused by DOX in hCPCs. We also suggest that these findings will help us to understand the mechanism by which DOX-induced damage is restored by GSH treatment.

**Material & Methods**

**Cell culture**

hCPCs were isolated from human heart tissues procured after surgical procedures as described in a previously modified protocol [7, 9, 27]. The Ethical Review Board of the Pusan National University Yangsan Hospital approved the protocol. To isolate hCPCs, the heart tissue specimens were minced into pieces of roughly 0.2 mm³ using fine scissors in a 60-mm petri dish on ice under aseptic conditions and were digested after transferring them into 50 mL tubes containing prewarmed 0.2% collagenase type II (Worthington, NJ, USA) solution in Ham's F12 medium (HyClone, GE Healthcare, Chicago IL, USA). The tubes were then incubated in a water bath at 37 °C for 1 h, with shaking every 10 min. Thereafter, single cardiac cells were passed through a 70 µm cell strainer and centrifuged at 1200 rpm for 3 min. hCPCs were cultured in Ham's F-12 medium containing 10% heat-inactivated fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific, Carlsbad, CA, USA), 1% penicillin/streptomycin (P/S, Welgene, Daegu, Republic of Korea), 0.2 mM of L-glutathione (Sigma-Aldrich #G4251, St. Louis, CA, USA), 20 ng/mL of recombinant human basic fibroblast growth factor (rb-FGF; PeproTech, Rocky Hill, NJ, USA), and 0.005 unit/mL of human erythropoietin (hEPO; R&D Systems, Minneapolis, MN, USA). The cultures were maintained at 37 °C in a 5% CO₂ humidified atmosphere.

**Cell Viability Assay**

The hCPCs viability assay was conducted using a CCK-8 kit (Dongin, CCK-3000, Seoul, South Korea), according to the manufacturer’s instructions. For analysis of cell proliferation ability, 10,000 cells/well were plated into each 96 well plate along with doxorubicin hydrochloride (Sigma-Aldrich, St. Louis, CA, USA) and glutathione at various concentrations and incubated for 24 h, after which the medium was changed to fresh drug-containing medium. After incubation, the CCK-8 solution was diluted to 1/10, and
the solution was changed to a medium with 100 µL. The plates were then incubated for 1 h. Absorbance was measured at 450 nm using a spectrophotometer (TECAN, Grodig, Austria). Each experiment was repeated at least three times.

**Apoptosis Assay**

The hCPCs apoptosis assay was performed using an Annexin V/PI kit (BD Pharmingen, #556547, San Diego, CA, USA). The hCPCs were pre-conditioned with doxorubicin and L-glutathione in Ham's F-12 medium. After 24 h incubation, hCPCs were harvested and washed with phosphate buffered saline (PBS), 2% FBS, and 200 µM EDTA. The pellets were suspended in 1X Annexin binding buffer with annexin V and propidium iodide (PI) according to the manufacturer's instructions. The assay was analyzed using fluorescence-activated cell sorting (FACS) (BD Accuri C6, BD Biosciences).

**Measurement Of Intracellular Ros Levels**

Intracellular ROS levels were measured using a H2DCFDA kit (Thermo Fisher Scientific, Carlsbad, CA, USA). Cells were harvested and washed with 2% FBS and 200 µM EDTA in PBS. hCPCs were incubated and after centrifugation at 2000 g, 3 min, the pellet was suspended in 5 µM H2DCFDA in PBS containing 2% FBS and 200 µM EDTA for 10 min at 37°C in 5% CO₂ atmosphere. After incubation, the cells were washed with PBS and analyzed by flow cytometry using the BD Accuri C6 software (BD Biosciences).

**Wound Healing Assay**

To investigate the migratory ability of the hCPCs, a 6 well plate was seeded at 200,000 cells/well and grown. Wounds were created by stroking the cells with a yellow pipette tip and the detached cells were washed with PBS. After 6 h of incubation, the migrated cells were observed under a light microscope (Olympus, Tokyo, Japan) using a 40x objective lens. The migrated area was measured using ImageJ software (Free software from National Institutes of Health) and was calculated using the following formula: percentage of migrated area: [(original scratched area – recovered scratched area) / original scratched area] × 100%.

**Migration Assay**

Migration assay was performed using a 24 well 8.0 µm polycarbonate trans-well chamber consisting of a permeable membrane (Corning Inc., Corning, NY, USA). For the assay, 500 µl of Ham's F-12 media culture medium was added below the cell permeable membrane, while 7,000 cells/100 µl in serum-free Ham's F-12 medium were plated on the upper chamber of the permeable membrane. After 24 h incubation, migrated cells were fixed with 4% paraformaldehyde and stained with 0.5% crystal violet at room temperature. The upper chamber was washed, and the top of the membrane was examined for cell
migration. The cells were then observed under an inverted microscope after mounting, and the number of cells was counted.

**Tube Formation Assay**

A tube formation assay was performed to assess the function of the hCPCs in the formation of blood vessel-like structures (tubes). 96-well plates were coated with 65 µL of Matrigel (BD Biosciences, San Diego, CA) and incubated at 37 °C for 30 min. After incubation, 7,000 cells were seeded into a 96 well plate coated with Matrigel, incubated for 7 h, and checked every hour to examine tube-forming ability. After incubation, the total tube length was measured using ImageJ software.

**Western Blotting**

After culturing in each group media, cells were lysed using RIPA lysis buffer (Thermo Fisher Scientific, Waltham, MA, USA) with a protease inhibitor (Thermo Fisher Scientific, Waltham, MA, USA). After 30 min of reaction at 4°C, a 13000 g 30 min centrifuge was used. After transferring the supernatant to a new 1.5 ml white e-tube, protein concentrations were quantified using a Bicinchoninic Acid Kit buffer (Thermo Fisher Scientific, Waltham, MA, USA). Equal amounts of protein were separated by an 8–15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene fluoride membrane (PVDF) (Millipore, Billerica, MA, USA). After the membrane was blocked in 5% skim milk for 1 h at room temperature, it was incubated overnight at 4 °C with primary antibodies specific for Cyclin D1 (1:200, Santa Cruz Biotechnology, Dallas, TX, USA; SC-8396), CDK4 (1:200, Santa Cruz, SC-56277), ERK (1:1000, Cell Signaling, 4695S), pERK (1:1000, Cell Signaling, 4376S), AKT (1:1000, Cell Signaling, 4691S), pAKT (S473) (1:1000, Cell Signaling, 4060S), pAKT (T308) (1:1000, Cell Signaling, 4056S), and β-actin (1:5000, Santa Cruz, SC-47778). Subsequently, the membranes were washed thrice with Tris-buffered saline containing 0.1% Tween 20 (TBST) and were incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. After washing the membranes again with TBST, the bands were visualized using Luminate Crescendo Western HRP Substrate (Millipore, Billerica, MA, USA) and an X-ray film. B-Actin was used as the loading control for western blotting.

**Doxorubicin-induced Cardiomyopathy Model**

Experiments were performed on 6–10-week-old male C57BL/6 mice maintained under a 12-h light/dark cycle in accordance with the regulations of the Pusan National University. All experiments were performed in accordance with the Pusan National University Institutional Animal Care and Use Committee (PNUIACUC). For the doxorubicin-induced cardiomyopathy model, C57BL/6 mice were intraperitoneally injected with doxorubicin (20 mg/kg) or glutathione (100 mg/kg). Body weight and survival rate were measured daily after the injection of doxorubicin and glutathione.
**Immunocytochemistry**

For immunocytochemistry, hCPCs were seeded at a density of 50,000 cells/well in a 12 well plate. After treatment with doxorubicin and glutathione for 24 h, differentiation was induced. After seven days, cells were washed twice with PBS and fixed with 4% paraformaldehyde for 10 min. Cell were permeabilized in 0.1% Triton X-100 in PBS (PBST) and incubated for 1 h at room temperature. The plate was then blocked with 5% normal goat serum in PBST for 1 h at room temperature. Alpha-smooth muscle antibody (1:200, Abcam) was diluted in 5% normal goat serum in PBST added to the wells. The plate was then incubated overnight at 4 °C, washed thrice with PBST, and incubated with Alexa Fluor 488 goat IgG anti-rabbit antibody (1:200, Invitrogen) for 1 h in the dark. After washing the cells twice, they were mounted using ProLong Diamond Anti-fade Mountant with 4',6’diamidino-2phenylindole (DAPI). The slides were analyzed using a Lionheart FX automated microscope (BioTek, Winooski, USA).

**Immunohistochemistry**

Five days after administration, mice were euthanized, and their heart tissue was retrogradely perfused with PBS and fixed with 4% paraformaldehyde overnight at 4 °C. Tissue sections (5 µm thickness) were subjected to immunohistochemistry with Masson's trichrome and Hematoxylin and Eosin (H&E) staining. Sections were examined using a Lionheart FX automated microscope (BioTek, Winooski, USA).

**Quantitative Real-time Pcr**

Total RNA was isolated using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) from the experimental cell groups and mouse heart tissue, according to the manufacturer’s specifications. Reverse transcription was performed on total RNA (1 µg) using the PrimeScript™ 1st strand cDNA Synthesis Kit (Clontech, TaKaRa 6110A). Real-time PCR was carried out in an Applied Biosystems 7500 Real-time PCR system (Thermo Fisher Scientific, Waltham, MA, USA) using SYBR Green PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA). For quantification, all samples were analyzed using the double delta Ct method \((2^{-\Delta\Delta Ct}}\). To confirm the value obtained from real-time PCR, we used a single-peak melting curve analysis.

**Statistical analysis**

Statistical analyses were conducted using two-tailed unpaired Student’s t-test and one-way analysis of variance (ANOVA) using GraphPad Prism software (GraphPad, Inc., La Jolla, CA, USA). Data are reported as mean ± standard deviation. Differences were considered statistically significant at \(p < 0.05\).

**Results**

**Effect of DOX and GSH in hCPCs viability**
To confirm whether hCPCs were damaged by DOX, they were exposed to 0.1, 0.2, 0.5, 1, 2, 4, and 5 µM DOX for 24 h. Cell viability was analyzed using the CCK-8 assay kit (Fig. 1A). The cell viability in the DOX group was lower than that in the control group. The GSH group showed higher cell viability in DOX group hCPCs. DOX (500 nM) was the preferred concentration based on morphological changes and cytotoxicity evaluation. The hCPCs were exposed to 0.1 and 1 mM concentrations of GSH for 24 h, out of which a concentration of 1 mM was found to prevent DOX-induced cytotoxicity in hCPCs (Fig. 1B). To confirm the recovery effect of GSH, we checked the cell viability of hCPCs exposed to 500 nM DOX and GSH at 0.1 and 1 mM concentrations and found that GSH indeed had a protective effect on damaged hCPCs (Fig. 1C). It also improved the cell confluency (Fig. 1D, E). Based on this, the cell cycle was confirmed to be western, and the cell numbers were counted (Fig. 1F, G). These data indicate that GSH can restore the hCPCs damaged by DOX (Fig. 1).

**Effect of GSH in apoptosis of hCPCs by DOX**

To determine whether cell death was caused by DOX-induced apoptosis, we evaluated hCPCs death using annexin V/PI staining (Fig. 2A). DOX treatment significantly increased the number of apoptotic cells and reduced the number of live cells. In contrast, the DOX with GSH-treated groups attenuated DOX-induced hCPCs apoptosis (Fig. 2B, C). These data suggest that the reduced cell viability shown in Fig. 1 was due to apoptosis.

**Effect of GSH on generated ROS by DOX in hCPCs**

ROS generation is well known to occur when cells are exposed to DOX. We investigated whether DOX-induced apoptosis in hCPCs is related to ROS generation. Although ROS increased in the DOX-treated group, co-treatment of hCPCs with DOX and GSH significantly decreased cellular ROS levels (Fig. 2D). A graph quantifying H2DCFDA is shown below (Fig. 2F). These data suggest that apoptosis caused by DOX in hCPCs is induced by ROS, which is restored by GSH.

**Effect of GSH on cell migration and tube formation capacity impaired by DOX in hCPCs**

To evaluate the function of hCPCs after exposure to DOX, we selected a 100 nM concentration of DOX that did not affect viability and exposed the cells treated with that concentration to GSH to conduct a wound-healing assay (Fig. 3A, B). The migration ability was reduced when the cells were treated with DOX alone but was restored in the group treated with DOX and GSH (Fig. 3C, D). Transwell migration assay was performed to confirm these results. The group exposed to GSH showed an increase in migration ability compared with the DOX (alone) group. Tube formation assays were performed to evaluate the other functions (Fig. 3E, F). Similar results were observed in tube formation experiments. These data suggest that GSH restores the functions of cell migration and tube formation impaired by DOX in hCPCs.

**Recovery effect of GSH related to pERK in hCPCs**

Based on the previous results, DOX-induced cytotoxicity was induced by ROS, and restored by antioxidant effect. We determined the mechanism by which GSH could be recovered. We detected various signaling
markers. These results suggested that while the amount of pERK in the cells increased when exposed to DOX, it decreased in the hCPCs after co-treatment with DOX and GSH, as confirmed by earlier studies [28, 29]. Upon exposure of the cells to the U0126, ERK inhibitor, there was an increase in the survival rate of the cells, which saw a decline in viability due to DOX (Fig. 4A, B). These data demonstrated that GSH was restored through ERK signaling.

**Effect of GSH and DOX in vivo**

To confirm whether GSH can inhibit DOX-induced cardiotoxicity, DOX (20 mg/kg, i. p.) or GSH (100 mg/kg, i. p.) was administered. The survival rate and body weight were measured daily (Fig. 6A). As a result, the DOX-treated group exhibited a lower survival rate and body weight compared to the control and DOX with GSH-treated groups (Fig. 5B, C). After harvesting the heart of the surviving mice on day 6 and sectioning the tissue, qRT-PCR, Masson trichrome staining (M&T), and hematoxylin and eosin (H&E) staining were performed. Bnp and Myh7 markers related to cardiotoxicity were identified at the mRNA level in the mouse heart. As a result, it was possible to confirm the decreased results in the DOX + GHS group compared with the increased results in the DOX group (Fig. 5D). In addition, when confirmed by immunohistochemistry, the heart tissue of the DOX + GSH group, which was relieved compared to the DOX group, in which fibrosis occurred at the injured site, was confirmed (Fig. 5E). These results suggested that GSH protects against DOX-induced cardiotoxicity.

**Discussion**

DOX, an anticancer agent, causes cell death by intercalating DNA, ROS generation, and mitochondrial dysfunction. Although DOX is an efficient drug for cancer treatment, it causes cardiotoxic side effects such as cardiomyopathy, heart failure, and arrhythmia [30, 31]. Therefore, many researchers have attempted to prevent these side effects, but there are no specific strategies to prevent and alleviate them. In reference, data support that DOX induces the production of oxidative free radicals and decreases the expression of antioxidant enzymes in the heart and mouse myocardial cells [32–34]. In this study, we identified GSH that enhanced hCPCs viability and function against DOX-induced cardiotoxicity (Fig. 1 and Fig. 4). It was confirmed that not only the viability of cells was restored, but also their function was restored, which suggests that cardiotoxicity caused by DOX treatment can be protected.

ROS are produced by normal metabolic processes and play crucial roles in cellular homeostasis, proliferation, and cell death. In this study, DOX caused apoptosis by the overproduction of ROS, leading to cardiotoxicity. To prevent DOX-induced ROS generation in hCPCs, we investigated compounds that exist in the body and are naturally produced rather than artificially. GSH is naturally synthesized and is present in almost all cells in the body. It also forms disulfide bonds with cysteine residues in proteins. Many researchers have studied the use of antioxidant drugs for DOX-induced side effects, but the mechanism of the protective effect of GSH has been overlooked [15, 17, 26, 35]. Thus, we hypothesized that GSH exerts a protective effect against DOX-induced cardiotoxicity. GSH has protective effects against cardiotoxicity in vitro and in vivo [36, 37]. Previous research [9, 38, 39] suggests that DOX treatment damages the heart. Our data support the importance of a protective effect against the proper modulation
of DOX-related hCPCs dysfunction. Treatment of hCPCs with GSH dramatically reduced DOX-induced apoptosis and cell death by enhancing CDK4 and Cyclin D1 activation (Fig. 1G). This was demonstrated by western blotting and Annexin V/PI staining assays (Fig. 2A-C). Cell viability was restored through the ERK signaling pathway, and the protective effect was also confirmed by the in vitro results (Fig. 4), including the migration ability and angiogenesis ability of hCPCs (Fig. 3), which were reduced due to the antioxidant effect of GSH. In addition to our previous in vitro results, the cardioprotective effect of GSH was confirmed in mouse experiments, in which the cardioprotective effect of GSH was demonstrated in relation to the induction of cardiotoxicity by DOX (Fig. 5). Previous studies have shown that DOX-induced cardiotoxicity induces apoptosis by regulating phosphorylated ERK (pERK). We found that the protective effect of GSH was related to the pERK pathway against cardiotoxicity, in vitro. Multiple studies support the fact that DOX increases pERK, leading to apoptosis [28, 40]. However, further research is needed to elucidate the mechanism by which pERK is involved in the protective effects of GSH.

In summary, we demonstrated that GSH protects against DOX-induced cardiotoxicity and ROS generation. In addition, this effect is regulated by pERK signaling, which is related to proliferation and viability. Our study suggests that modulating pERK using GSH is an efficient strategy to treat DOX-induced cardiotoxicity.

**Conclusion**

Treatment of hCPCs with DOX significantly increased the cellular ROS levels and apoptosis. However, pretreatment with GSH suppressed DOX-induced ROS generation and increased hCPC survival. GSH can be used to enhance the functions of hCPCs, such as tube formation and migration. GSH also contributes to the prevention of DOX-induced cardiomyopathy through the pERK pathway. Therefore, GSH could be used as an antioxidant drug for DOX-induced cardiomyopathy (Fig. 6).

**Declarations**

**Acknowledgements**

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**Data Availability**

The data used to support the findings of this study are available from the corresponding author upon request.

**Competing Interest**
The authors declare no competing financial interests.

References


Anthracycline cardiomyopathy is mediated by depletion of the cardiac stem cell pool and is rescued by restoration of progenitor cell function. *Circulation* 121: 276-292.


Figures

Figure 1

The effect of DOX and GSH in hCPCs viability. (A) hCPCs viability in various concentrations of DOX. (B) hCPCs viability in various concentrations of GSH. (C) hCPCs were exposed to DOX 500 nM, and GSH as a combination. (D) Cell morphology and (E) confluency after exposure to DOX alone and with GSH (F) Cell
number. (G) Western band detection related to cell cycle. Values are expressed as the mean ± standard derivation (S.D). *P<0.05, **P<0.01, ***P<0.001 as compared to the control group and #P<0.05 as compared to the DOX group.
Effect of GSH on apoptosis and ROS generation of hCPCs by DOX. (A) Apoptosis was measured by FACS and using annexin V/PI staining kit. (B), (C) Based on FACS, data were quantified at apoptotic cells and live cells. (D) ROS generation was measured by FACS and H2DCFDA. (E) It was quantified to ROS generation rates of FACS data. Values are expressed as the mean ± standard derivation (S.D). *P<0.05, **P<0.01, ***P<0.001 as compared to the control group and #P<0.05, ##P<0.01, ###P<0.001 as compared to the DOX group.
**Figure 3**

GSH enhances migration and tube forming capacity impaired by DOX in hCPCs. (A) The wound healing assay for select proper concentration had no effect on cell viability. (B) Data was quantified. (C) hCPCs was exposed to DOX 100 nM and GSH alone or together. The wound healing assay for the effect of GSH and DOX. (D) Quantification of wound healing assay. (E) The migration function by using trans-well migration kit. (F) Quantification of trans-well migration assay. (G) The tube formation ability of hCPCs exposed to DOX and GSH. (H) Quantification of tube length. Values are expressed as the mean ± standard derivation (S.D). *P<0.05, **P<0.01, ***P<0.001 as compared to the control group and #P<0.05, ##P<0.01, ###P<0.001 as compared to the DOX group. N.S.: non significance

**Table 1**

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**Figure 4**

Recovery effect of GSH on pERK in hCPCs. (A) The expression level of proteins related to ERK and AKT signaling. (B) Cell viability after DOX exposure and treatment with U0126 as an ERK inhibitor. Values are expressed as the mean ± standard derivation (S.D). *P<0.05 as compared to the DOX group

**Graph 1**

Cell viability (%) against different treatments.
Figure 5

Effect of GSH and DOX in vivo. (A) Schematic representation of in vivo experimental design. (B) Representative image of H&E staining and M&T staining at 6 days after IP injections of DOX and GSH. (C) Representative body weight and Kaplan-Meier estimator at 5 days after IP injections of DOX and GSH. (D) The cardiac tissues of each group were identified through immunohistochemistry. (E) Bnp and Myh7, markers of mRNA expression of cardiotoxicity were measured by qRT-PCR. Values of qRT-PCR are
expressed as mean ± standard error of the mean (S.E.M). *P<0.05, **P<0.01, ***P<0.001 as compared to the control group and #P<0.05 as compared to the DOX group

Figure 6

Proposed working model. GSH contributes to prevent DOX-induced cardiotoxicity though the pERK pathway