



Original Article

Protective effects of 7-hydroxyflavone on mitochondrial membrane potential and hydrogen peroxide-induced apoptosis in cardiomyocytes

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Submission : 20-Jan-2025

Revision : 17-Feb-2025

Acceptance : 12-Mar-2025

Web Publication : 05-Jun-2025

ABSTRACT

Objectives: The objective is to evaluate the protective effects of six coumarin derivatives against peroxide-induced cardiomyocyte damage and investigate their action mechanisms. **Materials and Methods:** Intracellular reactive oxygen species and mitochondrial membrane potential (MMP) were analyzed using dihydrorhodamine 123 and JC-1 combined with flow cytometry. Cell viability and apoptosis were assessed using WST-1 and lactate dehydrogenase analysis kits, respectively. The apoptotic signaling pathway was analyzed using a mouse apoptosis array kit. Cellular protein expression was detected using Western blotting. **Results:** Among the six coumarin derivatives tested, only 7-hydroxyflavone demonstrated the ability to protect cardiomyocytes from hydrogen peroxide (H₂O₂)-induced damage. Protein expression analysis revealed that 7-hydroxyflavone reduced cytochrome c release from the mitochondria and inhibited H₂O₂-induced activation of caspase-3. In addition, 7-hydroxyflavone maintained MMP stability in cardiomyocytes exposed to H₂O₂. **Conclusion:** 7-hydroxyflavone has potential as an effective antioxidant supplement for cardiac tissues. Further research is required to elucidate its pharmacokinetics and metabolic profile in humans to facilitate its therapeutic application.

KEYWORDS: 7-hydroxyflavone, Cardiomyocytes, Coumarin derivatives, Mitochondrial membrane potential, Reactive oxygen species

INTRODUCTION

Cardiomyocytes are particularly sensitive to hydrogen peroxide (H₂O₂)-induced damage owing to their high metabolic demand, unique membrane structure, and limited antioxidant defense mechanisms [1,2]. These cells rely heavily on mitochondrial oxidative phosphorylation to produce ATP, a process that generates reactive oxygen species (ROS), such as H₂O₂ and superoxide anions. The accumulation of ROS triggers oxidative stress, leading to cellular damage [3,4]. The membranes of cardiomyocytes and mitochondria are rich in unsaturated fatty acids, making them highly susceptible to peroxidation, which in turn disrupts membrane structure and functions, such as ion channel regulation and energy metabolism. In addition, peroxides can damage calcium-regulating proteins, leading to intracellular calcium imbalance, calcium overload, and ultimately apoptosis or necrosis [5-7]. Peroxides also oxidize proteins and DNA, thereby compromising cellular structures and function, as well as inhibiting enzyme activity and inducing genetic mutations [7,8]. Although cardiomyocytes possess intrinsic antioxidant defense systems, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), these

mechanisms can be depleted under severe oxidative stress and fail to fully protect cells [9-11]. Furthermore, adult cardiomyocytes have a limited regenerative capacity, making it difficult to restore their functionality after damage. Vulnerability to oxidative damage is a key pathological mechanism in cardiovascular diseases [12,13].

Coumarin is an aromatic compound naturally found in many plants that is known for its diverse biological activities and applications [14]. Its primary functions include antioxidant, anti-inflammatory, anticoagulant, anticancer, antimicrobial, and neuroprotective properties [15]. As a potent antioxidant, coumarin neutralizes free radicals, reducing oxidative stress and protecting cells and tissues, which is particularly beneficial for preventing cardiovascular and neurodegenerative diseases [16,17]. Its anti-inflammatory properties inhibit the production and release of inflammatory

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How to cite this article: Huang HL, Wang LK, Tsai FM. Protective effects of 7-hydroxyflavone on mitochondrial membrane potential and hydrogen peroxide-induced apoptosis in cardiomyocytes. Tzu Chi Med J 2025;37(4):403-11.

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| Quick Response Code:  | Website: www.tcmjmed.com |
| | DOI: 10.4103/tcmj.tcmj_21_25 |

mediators and alleviate chronic inflammatory diseases such as arthritis [18]. Coumarin exerts anticoagulant effects by inhibiting Vitamin K-dependent clotting factors and reducing the risk of blood clot formation. This property has been utilized in the development of anticoagulant drugs, such as warfarin, for treating deep vein thrombosis and stroke prevention [19]. The anticancer activity of coumarin includes the apoptosis induction, tumor cell proliferation inhibition, and tumor angiogenesis disruption, making it a promising candidate for cancer therapy [20]. In addition, their antimicrobial properties combat various bacterial, viral, and fungal infections, offering potential solutions for drug-resistant strains [21]. In terms of neuroprotection, coumarin and its derivatives reduce oxidative stress and inflammation in neural cells, demonstrating potential benefits for neurodegenerative diseases, such as Alzheimer's and Parkinson's [17].

Despite their beneficial properties, the direct application of coumarins is limited by drawbacks including low selectivity and potential toxicity. High doses of coumarin can cause hepatotoxicity and other adverse effects, raising safety concerns [22]. Furthermore, the rapid metabolism of coumarin *in vivo* reduces its efficacy, making it difficult to maintain stable therapeutic concentrations. Metabolic processes may also generate harmful intermediates and increase toxicity risk [23]. To address these issues, numerous coumarin derivatives with structural modifications tailored to target specific diseases and biological pathways have been developed, offering enhanced activity and broader applications. For example, warfarin, a classic coumarin derivative, is widely used as an anticoagulant to prevent thrombosis and cardiovascular diseases, with proven efficacy [24]. Other derivatives, such as 7-hydroxycoumarin and 6,7-dimethoxycoumarin, exhibit strong antioxidant and anti-inflammatory activities, making them suitable for the treatment of inflammation-related disorders [25,26]. Certain derivatives, such as 4-methylcoumarin and dihydrocoumarin, exhibit substantial anticancer activity by selectively inhibiting tumor cell growth while minimizing toxicity to normal cells [27,28].

Coumarin and its derivatives have been primarily studied for their roles in preventing thrombosis. For instance, warfarin, a Vitamin K antagonist, is used to prevent and treat various thrombotic conditions such as pulmonary embolism and cerebrovascular thrombosis. However, its specific mechanisms of action and efficacy in protecting cardiomyocytes remain unclear. Given the potent antioxidant properties of coumarins and their derivatives, we hypothesized that these compounds may protect cardiomyocytes from H₂O₂-induced damage. In this study, we aimed to evaluate the ability of several coumarin derivatives to protect cardiomyocytes from peroxide-induced damage. We assessed the effects of these derivatives on ROS generation and cell death in cardiomyocytes exposed to peroxide. In addition, we used apoptotic protein expression arrays to identify the molecular targets of the coumarin derivatives in mitigating oxidative damage. Finally, protein expression analysis and mitochondrial membrane potential (MMP) evaluation revealed that the coumarin derivative 7-hydroxyflavone reduced H₂O₂-induced MMP loss and cardiomyocyte death. Our findings demonstrate

the efficacy of 7-hydroxyflavone as a cardioprotective agent, highlighting its potential clinical applications beyond its known anticoagulant properties.

MATERIALS AND METHODS

Preparation of coumarin derivatives

Coumarin derivatives were prepared according to our previously published work [29]. The chemical structures of the six compounds analyzed in this study were illustrated previously [29]. The chemical and alternative names of the compounds are listed below: (i) compound 1: 7-[(2-methylbut-3-yn-2-yl)oxy]-2H-chromen-2-one, also known as 7-(1,1-dimethyl-propynyloxy)-coumarin; (ii) compound 2: 8,8-dimethyl-2H,8H-pyrano[2,3-f]chromen-2-one, also known as Seselin; (iii) compound 3: 8,8-dimethyl-2H,8H-pyrano[3,2-g]chromen-2-one, also known as Corylin; (iv) compound 4: 7-hydroxy-3-phenyl-4H-chromen-4-one; (v) compound 5: 2-(2,2-dimethyl-2H-chromen-6-yl)-7-hydroxychroman-4-one, also known as 7-hydroxyflavone; and (vi) compound 6: 7-hydroxy-2',2'-dimethyl-2'H,4H-[3,6'-bichromen]-4-one.

Cell culture

Rat H9c2 (2-1) cardiomyocytes were purchased from Bioresource Collection and Research Center (Hsinchu City, Taiwan). HL-1 cardiac muscle cell line was obtained from Sigma-Aldrich (St. Louis, MO, USA). H9c2 cells were cultured as described in a previous study [30]. Briefly, cells were maintained in Dulbecco's modified Eagle's medium containing 4 mM L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, and 10% fetal bovine serum (FBS). HL-1 cells were cultured in a commercially available medium (Claycomb Basal Medium) supplemented with 2 mM L-glutamine, 0.1 mM norepinephrine, and 10% FBS. H9c2 and HL-1 cells were incubated in an atmosphere containing 5% carbon dioxide. For this study, early-passage cells (passages 4–7) were used. Routine mycoplasma testing was conducted during the cell culture period to confirm the absence of contamination.

Measurement of reactive oxygen species and mitochondrial membrane potential activity

Dihydrorhodamine 123 (DHR123) and JC-1 were used to measure intracellular ROS production and MMP, respectively. The measurement methods used were based on previous studies [30,31]. Briefly, H9c2 and HL-1 cells (1 × 10⁶ cells) were seeded in 10-cm culture dishes. After serum-free culture and treatment with different concentrations of coumarin derivatives for 24 h, the cells were exposed to 100 μM H₂O₂ and simultaneously treated with 2.5 μM DHR123 or 200 μM JC-1 for 30 min to 1 h. Trypsinized cells were filtered through a membrane with 30 μm pore size and analyzed using a flow cytometer (CytoFLEX S, Beckman Coulter, IN, USA). ROS analysis was performed at excitation/emission wavelengths of 488/525 nm, while MMP was analyzed at 488/525 nm and 488/585 nm.

Measurement of cell viability and cell death

Cell viability and death were assessed as previously described [30]. Briefly, H9c2 and HL-1 cells (1 × 10⁵ cells) were seeded in 6-well culture plates. After serum-free culture and treatment with different concentrations of coumarin derivatives for 24 h, the cells were exposed to 100 μM H₂O₂ for

6 h. Cell viability was measured using WST-1 reagent (Roche Diagnostics, Mannheim, Germany), and cell death was evaluated by quantifying lactate dehydrogenase (LDH) release using a cytotoxicity detection kit (Roche Diagnostics).

Analysis of apoptosis array

Differential protein expression associated with apoptosis in HL-1 cells was analyzed using a mouse apoptosis array kit (Proteome Profiler; R and D Systems, Minneapolis, MN, USA). After treatment with coumarin derivatives for 24 h and subsequent exposure to 100 μM H_2O_2 for 6 h, cells were lysed with the lysis buffer provided in the kit (Lysis Buffer 17). Intracellular proteins were collected and quantified using a Bio-Rad protein assay. For each array, 300 μg of total protein was used. Protein expression was visualized using the Chemi Reagent Mix included in the kit and imaged with a luminometer (Bio-Rad ChemiDoc XRS + system).

Isolation of mitochondrial proteins

Mitochondria were isolated from the cultured cells (Mitochondria Isolation Kit for Cultured Cells; Thermo Fisher Scientific, Waltham, MA, USA). The isolation was performed according to the manufacturer's instructions.

Western blotting

Western blot analysis was performed according to previously described methods [32]. Briefly, H9c2 and HL-1 cells (1×10^6 cells) were seeded in 10-cm culture dishes and treated for 24 h, followed by exposure to 100 μM H_2O_2 for 6 h. The cell lysates were collected for protein analysis. The antibodies used in this study included those against caspase-3, cleaved caspase-3 (Asp175), Bcl-2 (D17C4), Bad, and cytochrome c (13F6) from Cell Signaling Technology (Beverly, MA, USA), and actin antibodies from Sigma-Aldrich.

Statistical analysis

All experiments were conducted in triplicate, and data are expressed as the mean \pm standard deviation. Statistical analysis was conducted using one-way analysis of variance, followed by Dunnett's *post hoc* test. Statistical significance was defined as $P < 0.05$.

RESULTS

Protective effects of selected coumarin derivatives against hydrogen peroxide-induced cardiomyocyte injury

Using H9c2 and HL-1 cells as experimental models, we evaluated the effects of six different coumarin derivatives (20 μM each) on ROS production induced by 100 μM H_2O_2 treatment for 1 h. As shown in Figure 1, among the tested compounds, only compound 5 significantly inhibited H_2O_2 -induced ROS production in H9c2 [Figure 1a] and HL-1 [Figure 1b] cardiomyocytes.

In addition to analyzing ROS production, we assessed whether the coumarin derivatives could protect against H_2O_2 -induced damage in cardiomyocytes. H_2O_2 treatment reduced cell viability by 57.0% in H9c2 cells and 44.3% in HL-1 cells [Figure 1c and e]. Consistent with the ROS findings, only compound 5 significantly mitigated the H_2O_2 -induced reduction in cell viability,

restoring viability by 33.0% in H9c2 cells and 35.1% in HL-1 cells [Figure 1c and e].

We also assessed H_2O_2 -induced cell death by measuring LDH release as an indicator of cytotoxicity. H_2O_2 increased cell death by 60.8% in H9c2 cells and 33.7% in HL-1 cells [Figure 1d and f]. Similarly, only compound 5 effectively reduced cell death, decreasing it by 48.8% in H9c2 cells and 41.2% in HL-1 cells, respectively [Figure 1d and f].

Protective effects of 7-hydroxyflavone against hydrogen peroxide-induced cardiomyocyte injury

The chemical structure of compound 5, which was identified as 7-hydroxyflavone, is shown in Figure 2a. Treatment with 7-hydroxyflavone (5–50 μM) alone did not affect cell viability in either H9c2 or HL-1 cells (data not shown). The antioxidant capacity of various concentrations of 7-hydroxyflavone in cardiomyocytes was tested. Dose-dependent reductions in H_2O_2 -induced ROS production were observed in H9c2 [Figure 2b] and HL-1 [Figure 2c] cells when treated with increasing doses of 7-hydroxyflavone.

At higher doses (20–50 μM), 7-hydroxyflavone alleviated the H_2O_2 -induced decline in cell viability [Figure 2d] and attenuated cell death [Figure 2e] in H9c2 cells. Similar protective effects were observed in HL-1 cells [Figure 2f and g]. However, even at a high dose of 50 μM 7-hydroxyflavone, complete protection against H_2O_2 -induced damage was not achieved in either H9c2 or HL-1 cells, as cell viability still decreased by 14.2% and 8.6%, respectively.

7-hydroxyflavone reduces hydrogen peroxide-induced upregulation of cleaved caspase-3 and cytochrome c

To explore the molecular pathways involved in the protective effects of 7-hydroxyflavone, we performed an apoptosis array analysis in HL-1 cells. The results showed that H_2O_2 treatment increased the expression of apoptosis-related proteins, including Bad, Bcl-x, cleaved caspase-3, cytochrome c, and SMAC/Diablo. Notably, 7-hydroxyflavone treatment reduced the expression of cleaved caspase-3 and cytochrome c [Figure 3a]. Treatment with 7-hydroxyflavone alone had no significant effect on the expression of apoptosis-related proteins [Figure 3a].

Western blot analysis further confirmed these findings in H9c2 and HL-1 cells. H_2O_2 increased cleaved caspase-3 expression while decreasing total caspase-3 levels in H9c2 cells. Treatment with 20–50 μM 7-hydroxyflavone effectively inhibited H_2O_2 -induced cleaved caspase-3 expression [Figure 3b]. Similar effects were observed in the HL-1 cells [Figure 3c]. However, the expression patterns of Bad differed between the two cell lines. In H9c2 cells, treatment with 20–50 μM 7-hydroxyflavone inhibited the H_2O_2 -induced upregulation of Bad [Figure 3b and c]. By contrast, in HL-1 cells, 7-hydroxyflavone treatment did not alter the H_2O_2 -induced increase in Bad expression, which was consistent with the array results. A similar trend was observed for Bcl-2 expression, as 7-hydroxyflavone treatment did not reverse the H_2O_2 -induced downregulation of Bcl-2 in HL-1 cells [Figure 3c].

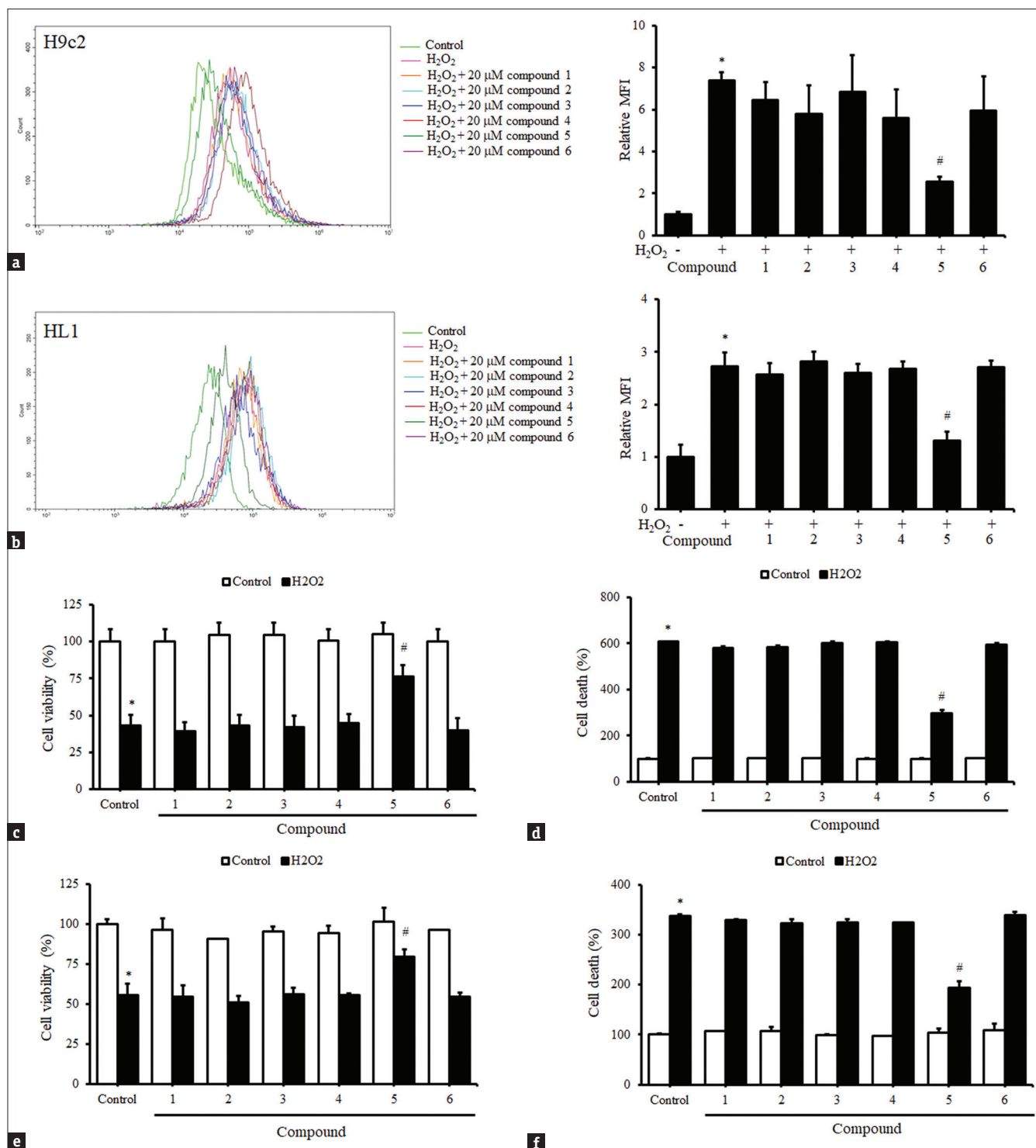


Figure 1: Effects of six coumarin derivatives on hydrogen peroxide (H₂O₂)-induced reactive oxygen species (ROS) production, decreased cell viability and cell death in cardiomyocytes. H9c2 cells (a) and HL1 cells (b) were treated with 20 μM of coumarin derivatives for 24 h, followed by 100 μM H₂O₂ treatment for 1 h. Cells were stained with DHR123 dye and analyzed for ROS generation using flow cytometry. Data are presented as the fold change in MFI values compared to the untreated control group. H9c2 (c and d) and HL1 cells (e and f) were treated with 20 μM of coumarin derivatives for 24 h, followed by 100 μM H₂O₂ treatment for 6 h. Cell viability (c and e) was assessed using WST-1 reagent, and cell death (d and f) was detected by lactate dehydrogenase release. Data are presented as percentages relative to the untreated control group. **P* < 0.05 compared to the control group. #*P* < 0.05 compared to cells treated with H₂O₂. H₂O₂: Hydrogen peroxide

To further assess cytochrome c activation, a mitochondrial isolation kit was used to analyze its intracellular distribution. First, successful mitochondrial fractionation was confirmed in untreated H9c2 cells (data not shown). In addition, H₂O₂

was found to decrease mitochondrial cytochrome c levels while increasing cytosolic expression, indicative of a loss of MMP [Figure 3d]. Treatment with 20–50 μM 7-hydroxyflavone reduced the cytosolic expression of cytochrome c in

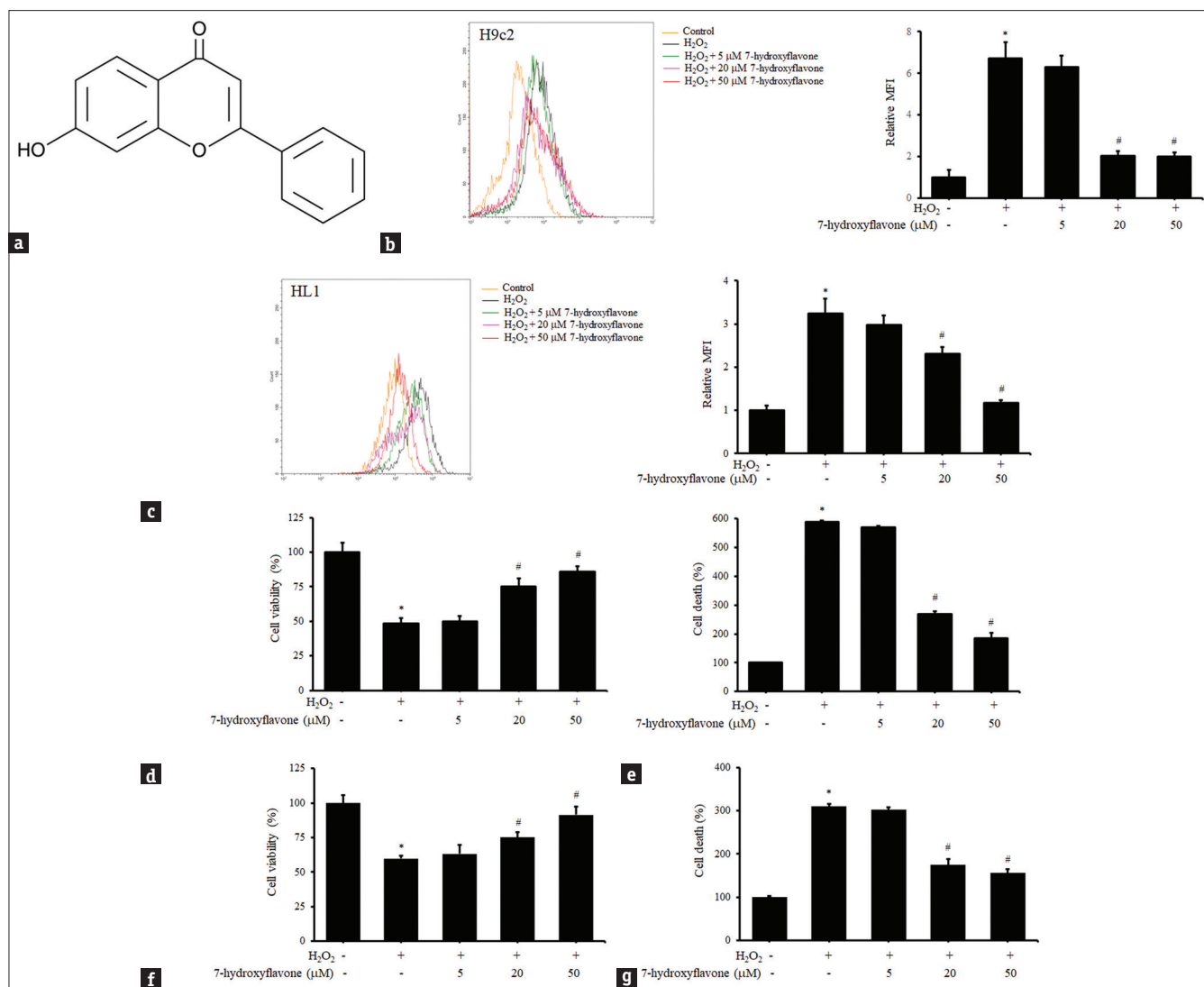


Figure 2: 7-hydroxyflavone inhibits hydrogen peroxide (H₂O₂)-induced reactive oxygen species (ROS) production, reduction in cell viability and cell death in cardiomyocytes. Chemical structure of 7-hydroxyflavone (a). H9c2 (b) and HL1 cells (c) were treated with 5–50 μM of 7-hydroxyflavone for 24 h, followed by 100 μM H₂O₂ treatment for 1 h. The cells were stained with DHR123 dye and analyzed for ROS generation using flow cytometry. Data are presented as the fold-change in MFI values compared to those of the untreated control group. H9c2 (d and e) and HL1 cells (f and g) were treated with 5–50 μM of 7-hydroxyflavone for 24 h, followed by 100 μM H₂O₂ treatment for 6 h. Cell viability (d and f) was assessed using WST-1 reagent, and cell death (e and g) was detected by lactate dehydrogenase release. Data are presented as percentages relative to the untreated control group. **P* < 0.05, compared with the control group. #*P* < 0.05, compared with cells treated with H₂O₂. H₂O₂: Hydrogen peroxide

H9c2 cells [Figure 3d]. Comparable results were observed in HL-1 cells, where 7-hydroxyflavone suppressed H₂O₂-induced cytochrome c expression in the cytosol [Figure 3e].

Finally, we analyzed apoptosis using propidium iodide staining. The results showed that H₂O₂ treatment increased the proportion of the sub-G1 cell population in H9c2 and HL-1 cells to 78.41%–83.99%. Treatment with 20–50 μM 7-hydroxyflavone effectively reduced the H₂O₂-induced increase in the sub-G1 cell population in both cell lines [Figure 3f].

7-hydroxyflavone alleviates hydrogen peroxide-induced mitochondrial membrane potential loss

The results from the protein array and western blot analyses suggested that 7-hydroxyflavone mitigates H₂O₂-induced changes in MMP. To confirm this, we used the MMP dye JC-1.

As shown in Figure 4, H₂O₂ treatment caused a significant loss of MMP in H9c2 cells. Treatment with 20–50 μM 7-hydroxyflavone significantly attenuated this loss [Figure 4a]. Similarly, 7-hydroxyflavone protected HL-1 cells from H₂O₂-induced MMP damage [Figure 4b].

DISCUSSION

Coumarin derivatives are known for their strong antioxidant properties, primarily owing to their phenolic hydroxyl groups and aromatic rings, which scavenge free radicals, reduce ROS accumulation, and inhibit lipid peroxidation. Furthermore, these compounds can enhance the activity of antioxidant enzymes such as SOD and GPx, thereby reducing oxidative stress and protecting cells and tissues from damage. All six tested coumarin derivatives possessed structural elements, including phenolic hydroxyl groups and aromatic rings.

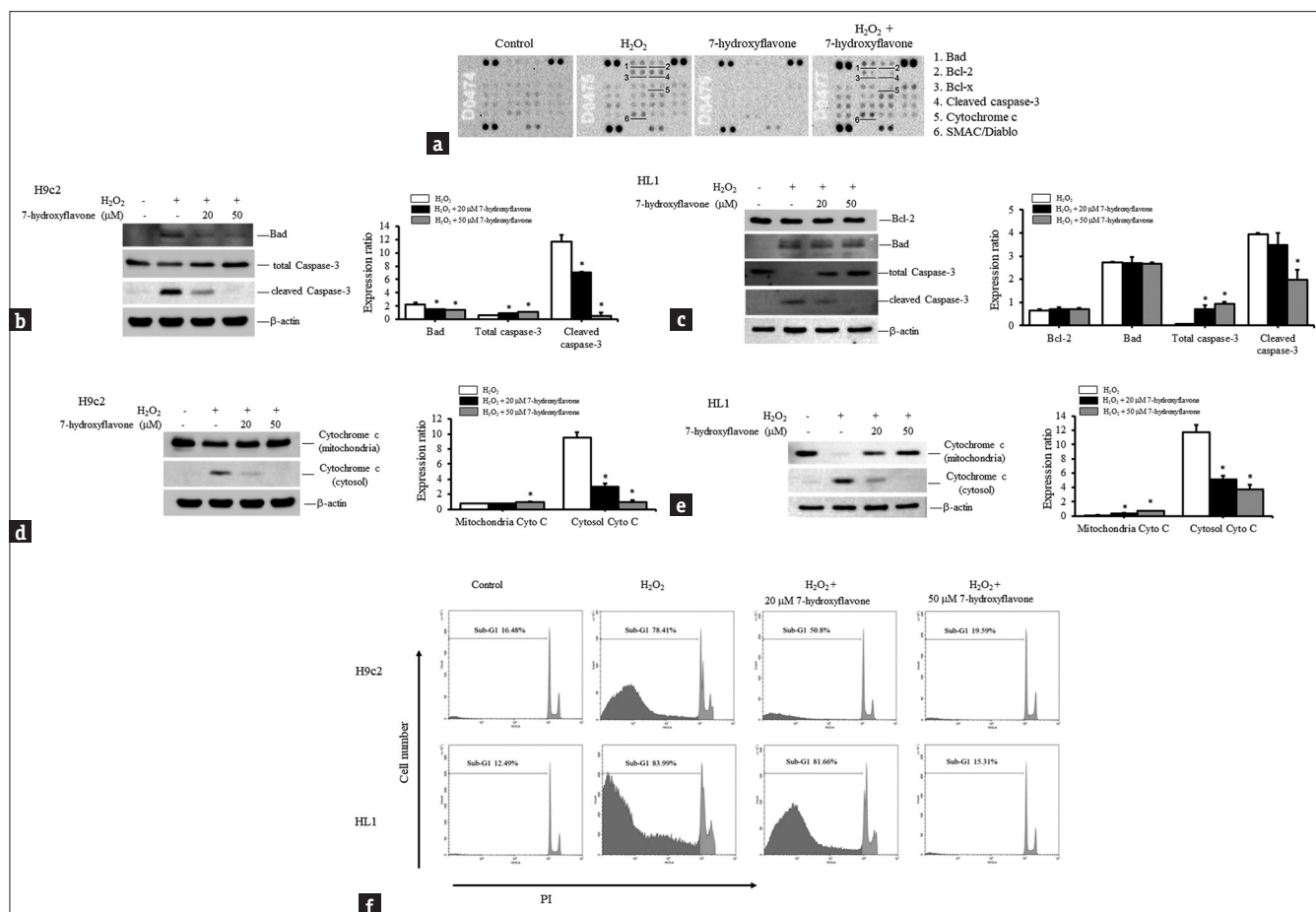


Figure 3: Effects of 7-hydroxyflavone and hydrogen peroxide (H_2O_2) on the expression of apoptosis-related proteins in cardiomyocytes. HL1 cells were treated with 20 μM of 7-hydroxyflavone for 24 h, followed by 100 μM H_2O_2 treatment for 6 h. Cell lysate was collected, and protein expression was analyzed using a mouse apoptosis array kit (a). H9c2 (b and d) and HL1 cells (c and e) were treated with 20–50 μM of 7-hydroxyflavone for 24 h, followed by 100 μM H_2O_2 treatment for 6 h. The expression of Bcl-2, Bad, caspase-3 and cytochrome c was analyzed using western blotting. H9c2 and HL1 cells were treated with 20–50 μM of 7-hydroxyflavone for 24 h, followed by 100 μM H_2O_2 treatment for 24 h. Cells were stained with propidium iodide and analyzed using flow cytometry. (f) The bar graphs represent fold changes relative to the untreated control group. * $P < 0.05$ compared to the control group treated with H_2O_2 . H_2O_2 : Hydrogen peroxide

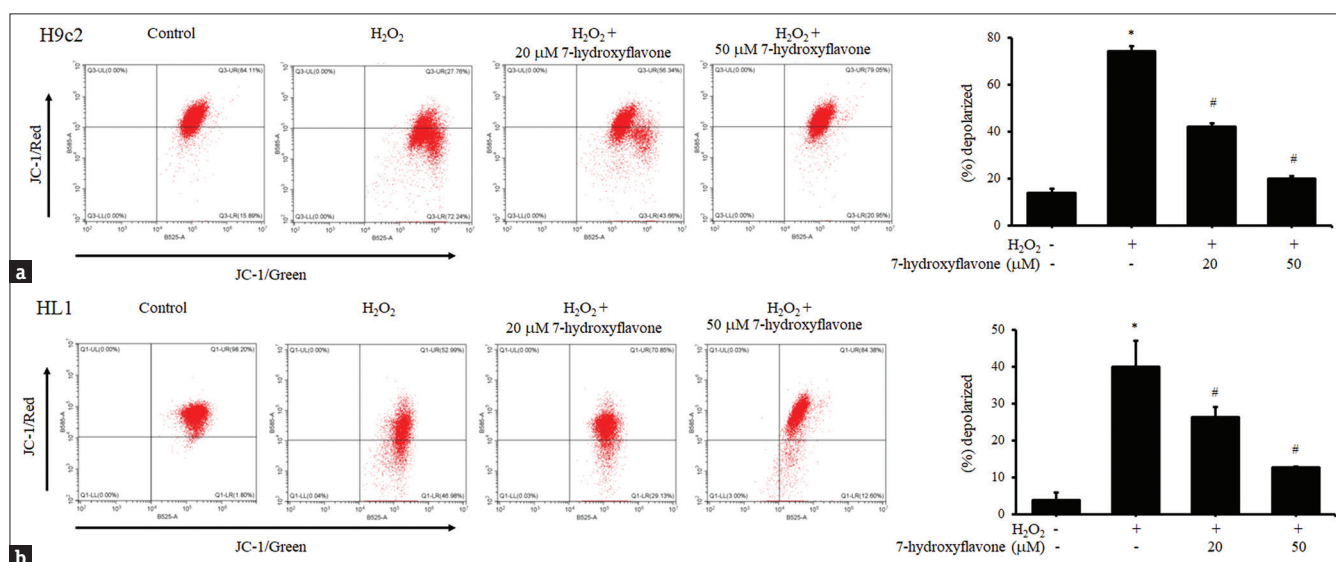


Figure 4: Effects of 7-hydroxyflavone and hydrogen peroxide (H_2O_2) on mitochondrial membrane potential in cardiomyocytes. H9c2 (a) and HL1 cells (b) were treated with 20–50 μM of 7-hydroxyflavone for 24 h, followed by 100 μM H_2O_2 treatment for 30 min. Cells were stained with JC-1 dye and analyzed using flow cytometry. Data are presented as the percentage relative to the untreated control group. * $P < 0.05$ compared to the control group. # $P < 0.05$ compared to cells treated with H_2O_2 . H_2O_2 : Hydrogen peroxide

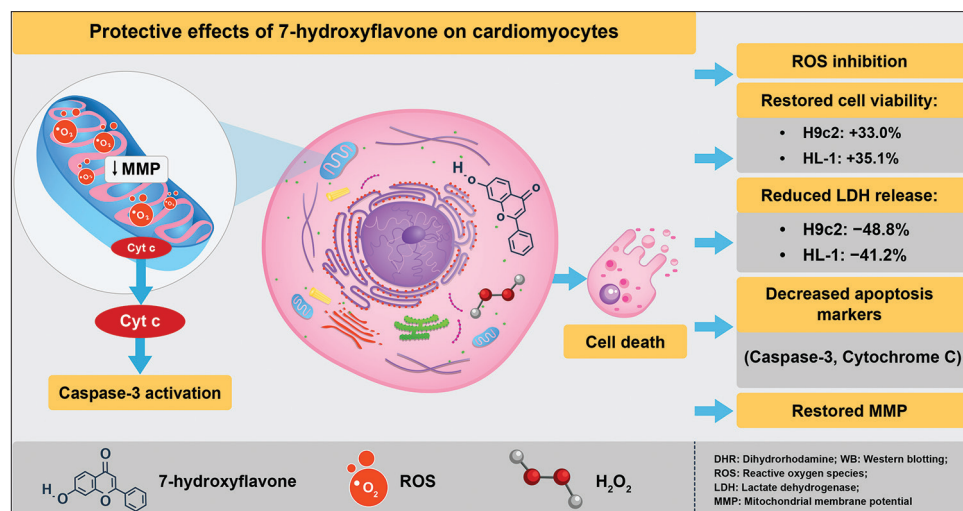


Figure 5: Schematic diagram illustrating the mechanism by which 7-hydroxyflavone protects cardiomyocytes from oxidative damage by preserving MMP and inhibiting apoptosis

However, among these, only 7-hydroxyflavone exhibited protective effects against oxidative damage in cardiomyocytes. Therefore, in addition to the inherent structural effects of coumarin derivatives, interactions with intracellular proteins or receptors may play a role in their activity. Orphan nuclear receptor 4A1 has been identified as a receptor for both 7-hydroxyflavone and flavones in cells [33]. Furthermore, the orphan nuclear receptor 4A1 plays a crucial role in maintaining the MMP and regulating cell death under conditions of hypoxia and reoxygenation in cardiomyocytes [34-36]. Therefore, we hypothesized that orphan nuclear receptor 4A1 may be involved in the protective effects of 7-hydroxyflavone in cardiomyocytes. However, further investigation is needed to obtain conclusive evidence.

The loss of MMP has profound effects on cardiomyocyte function and may contribute to the development of various heart diseases. Mitochondria, which constitute 20%–35% of the cardiomyocyte volume, serve as the cell's powerhouse, supplying the ATP necessary for contraction [37]. The MMP is the driving force for ATP synthesis, and its loss directly affects ATP production, leading to an insufficient energy supply for cardiomyocytes. When MMP decreases, the electron transport chain is impaired, leading to increased ROS production. Excess ROS damages mitochondria and cellular structures, exacerbates mitochondrial dysfunction, and creates a vicious cycle [38]. In addition, the loss of MMP can trigger apoptotic pathways, resulting in cardiomyocyte death, which is particularly evident in cardiac ischemia-reperfusion injury [39].

In this study, we identified the potential signaling pathways involved in the protective effects of 7-hydroxyflavone on cardiomyocytes using a mouse apoptosis array. Although we found that mitochondrial cytochrome c release and the activation of caspase-3 pathways were involved, these are common pathways activated during oxidative damage. Compared to the 21 proteins detected in the commercial mouse apoptosis array (https://www.rndsystems.com/products/roteome-profiler-mouse-apoptosis-array_ary031), the human apoptosis array detected 35 different protein targets (<https://www.rndsystems.com/products/>

roteome-profiler-human-apoptosis-array-kit_ary009). Owing to the limitations of species-specific cardiomyocytes and available kits, we were only able to identify the preliminary targets of 7-hydroxyflavone action in cardiomyocyte mitochondria. Further research is needed to clarify how 7-hydroxyflavone regulates apoptotic pathways.

In addition to these findings, previous studies have highlighted the antioxidant effects of 7-hydroxyflavone. For instance, 7-hydroxyflavone has been found to enhance the cellular antioxidant capacity by activating the ERK/Nrf2/HO-1 signaling pathway, thereby protecting renal cells from nicotine-induced oxidative damage [40]. In addition, 7-hydroxyflavone has shown therapeutic potential in improving nonalcoholic fatty liver disease, likely due to its antioxidant properties [41]. *In vitro* experiments have shown that 7-hydroxyflavone can activate HO-1 expression, reduce oxidative stress, and inhibit caspase-3 and PARP activation, thereby decreasing cell damage [42]. Moreover, 7-hydroxyflavone has been found to interact with DNA, potentially protecting it from oxidative damage through its antioxidant properties [43]. Our study revealed that 7-hydroxyflavone effectively inhibited H₂O₂-induced ROS production, MMP loss, and apoptosis in both H9c2 and HL-1 cells [Figures 2 and 4]. In addition, 7-hydroxyflavone suppressed H₂O₂-induced Bad upregulation in H9c2 cells but had no effect on Bad expression in HL-1 cells [Figure 3]. As previously discussed, although 7-hydroxyflavone may scavenge free radicals through its aromatic rings, its primary protective mechanism appears to involve pathways that preserve mitochondrial function. The ERK/Nrf2/HO-1 signaling pathway is primarily associated with cytoplasmic signaling and nuclear activation, distinct from mitochondrial pathways [44]. This suggests that 7-hydroxyflavone exerts its antioxidant effects through multiple signaling mechanisms. Based on our results, we hypothesize that in cardiomyocytes, the protective effects of 7-hydroxyflavone against H₂O₂-induced damage are primarily mediated through mitochondrial pathways.

The role of antioxidant drugs in the maintenance of cardiac function has attracted widespread attention. These

drugs primarily reduce the adverse effects of oxidative stress on cardiomyocytes improving cardiac health and preventing related diseases. Many antioxidants, such as coenzyme Q10 [45,46], Vitamin E [47], and Omega-3 fatty acids [48], are considered safe within recommended dosage ranges and are suitable for daily heart health maintenance. However, data regarding the antioxidant effects of coumarin derivatives in human clinical trials are limited. Most studies investigating the effects of antioxidants on cardiomyocytes use cell lines [49-51], whereas *in vivo* studies typically assess overall cardiac function by analyzing myocardial contractile dysfunction [52], making it challenging to elucidate the detailed mechanisms of drug action. Our study confirms the potential antioxidant effects of 7-hydroxyflavone in cardiomyocytes; however, further research is needed to determine whether these effects can be replicated *in vivo* or translated into clinical applications.

CONCLUSION

In this study, we demonstrated that among the coumarin derivatives tested, 7-hydroxyflavone was the only compound that effectively protected cardiomyocytes from oxidative damage. Furthermore, 7-hydroxyflavone was found to primarily exert its protective effects by maintaining the MMP and inhibiting oxidative stress-induced apoptosis [Figure 5]. These findings strongly support the potential of 7-hydroxyflavone as an effective antioxidant with broad biological activity in cardiomyocytes. However, its precise mechanisms of action and effects in various biological systems require further study to establish a solid theoretical foundation for its medical and health applications.

Acknowledgments

The authors would like to thank the Core Laboratory of the Taipei Tzu Chi Hospital for support.

Data availability statement

All data generated or analyzed during this study are included in the article, further inquiries can be directed to the corresponding author.

Financial support and sponsorship

This work was supported in part by grants from the Taipei Tzu Chi Hospital through grants from the Buddhist Tzu Chi Medical Foundation under the numbers TCRD-TPE-113-20.

Conflicts of interest

There are no conflicts of interest.

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