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Research Article

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PROTECTIVE EFFECT OF PEONIDIN - ANTHOCYANIDIN CLASS FLAVONOID AGAINST DOXORUBICIN-INDUCED TOXICITY IN H9C2 CARDIOMYOBLAST CELL LINES

Jyothirmai Namathoti, Rajeshwari Pasupula*

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ABSTRACT
Background: This study investigates the cardioprotective properties of peonidin against cytotoxicity induced by doxorubicin (DOX) in H9c2 cardiomyocytes using both in vitro and in silico methods. H9c2 cells were exposed to DOX alone as well as in combination with different concentrations of peonidin for various time durations. **Methodology:** Cytoprotection was studied using MTT assay for viability, LDH leakage, SOD activity, lipid peroxidation, and ROS content. Furthermore, molecular docking was also performed to analyze the binding affinity of peonidin with angiotensin-converting enzyme (ACE) and endothelin-converting enzyme-1 (ECE-1), utilizing captopril as a control. **Results and Discussion:** DOX drastically inhibited cell viability, whereas peonidin co-treatment maintained it dose dependently, restoring it to 99.74% at 150 µg/mL after 72 h. LDH release through DOX-triggered membrane disruption was alleviated from 175.84% to 78.40% by peonidin. ROS levels were also decreased from 191.13% (DOX) to 61.29% by peonidin, reflecting robust antioxidant activity. Lipid peroxidation was strongly inhibited, and SOD activity, decreased by DOX (36.59±0.306 AU), was restored close to control levels (97.85±0.313 AU) by peonidin. Docking studies indicated that peonidin exhibited a superior binding affinity with ACE (-95.72 kcal/mol) and ECE-1 (-78.08 kcal/mol) compared to captopril, characterized by good van der Waals and hydrogen bonding interactions. **Conclusion:** Peonidin potently mitigates DOX-induced oxidative injury in cardiomyocytes, showing great promise as a natural cardioprotective agent, as evidenced by both biochemical assays and molecular docking studies against ACE and ECE-1.

INTRODUCTION

The cardiac injury caused by doxorubicin, which is a widely utilized anthracycline antibiotic in cancer chemotherapy, is known as Doxorubicin-induced myocardial damage. Doxorubicin is effectively used to treat various malignancies; however, its clinical utility is significantly limited due to its cardiotoxic effects. This injury manifests mostly as cardiomyopathy that can evolve to congestive heart failure,

Department of Pharmacology, College of Pharmacy, Koneru Lakshmaiah Education Foundation, Green Fields, Vaddeswaram, Guntur-522302, Andhra Pradesh, India.

For Correspondence: **rajeswaripasupula@kluniversity.in**

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sometimes even years after therapy [1]. The ROS formation in cardiac cells causes oxidative stress and secondary damage to cellular components, including lipids, proteins, and DNA. The cumulative dose of doxorubicin was a very critical factor in the extent of myocardial damage, with higher doses correlating with increased risk [2]. Despite its efficacy in treating cancer, the risk of doxorubicin-induced myocardial damage necessitates careful monitoring of cardiac function during and after chemotherapy, as well as the exploration of cardioprotective strategies to mitigate these adverse effects [3].

Cardioprotective activity refers to the ability of specific compounds or interventions to protect the heart muscle (myocardium) from injury and maintain its function, particularly in situations that may cause cardiovascular damage or disease. The activity was essential in minimizing the effects of diverse types of stress on the heart, such as ischemia (limited blood flow), oxidative stress, inflammation, and drug toxicity, such as that caused by doxorubicin. The cardioprotective drugs act through various mechanisms, including minimizing oxidative stress by neutralizing free radicals, enhancing the efficacy of energy metabolism in cardiac cells, stabilizing cell membranes, and suppressing inflammatory pathways that can exacerbate heart damage [4]. These drugs can also enhance the survival and regeneration of cardiac cells, minimize apoptosis (programmed cell death), and enhance overall cardiac function. Natural products, such as flavonoids and polyphenols, as well as certain drugs, have demonstrated remarkable cardioprotective activity in both clinical and experimental settings. Cardioprotective activity and awareness are crucial in the creation of therapeutic methods to avert or limit cardiac injury, especially in subjects under high risk of cardiovascular events, including chemotherapy subjects, victims of heart attack, or patients with chronic cardiovascular diseases [5].

Anthocyanidin-type compounds are flavonoids present in plants and have potent cardioprotective activity. Fundamentally, their high antioxidative ability quenches ROS and reduces cardiovascular harm caused by oxidative stress. Anthocyanidins significantly help prevent atherosclerosis, the primary cause of coronary artery disease, by protecting endothelial cells and inhibiting the oxidation of LDL cholesterol [6]. Additionally, the compounds have potent anti-inflammatory actions by blocking pro-inflammatory cytokines and enzymes, which act as a protective mechanism against inflammation in the cardiovascular system [7]. Such anti-inflammatory activity decreases the risks of myocardial infarction and atherosclerosis. Peonidin belongs to the anthocyanidin class of flavonoid compounds, which are plant coloring pigments with the ability to generate red, purple, and blue colors in fruits, flowers, and vegetables.

A critical examination of the available research studies reveals that no previous studies have been conducted to assess the cardioprotective ability of peonidin. Thus, this study aims to determine the cardioprotective effect of peonidin against doxorubicin toxicity in H9c2 cardiomyoblast cell lines.

MATERIALS AND METHODS

Chemicals and reagents

The H9C2 rat cardiac myoblasts, derived from embryonic rat heart tissue, were purchased from Sigma-Aldrich, Bangalore, India. Fetal Bovine Serum (FBS) and Dulbecco's Modified Eagle Medium (DMEM) are purchased from Thermo Fisher Scientific. Doxorubicin (DOX), Malondialdehyde (MDA), *3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide* (MTT), Cytotoxicity Assay Kit, *2′,7′-dichlorofluorescein diacetate* (DCF-DA), thiobarbituric acid reactive substances (TBARS) assay kit, β-methylphenethylamine (β-ME), phenylmethylsulfonyl fluoride (PMSF), monosodium salt of *2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfo-phenyl)-2H-tetrazolium* (WST), DMSO and butylated hydroxytoluene (BHT) are purchased from Sigma Aldrich, Bangalore, India.

Cell Culture and Treatment

The cryopreserved H9C2 cells were collected from liquid nitrogen and immediately thawed to 37°C using a water bath [8]. They were then cultured in T75 cm^2 culture flasks containing DMEM supplemented with 10% FBS. The cultured cells were incubated for 48 hours, after which they were counted and seeded in a 96-well plate at a density of 2×10^5 cells per well. The seeded plates were incubated at 37°C in a humidified atmosphere with 5% CO_2. Then, the cells were exposed to peonidin at 50, 100, and 150 µg/mL along with Doxorubicin (DOX), and the experimental setup consisted of five groups:

1. **Control group** – Cells were cultured in DMEM for 24 hours and then treated with saline for another 24 hours.
2. **DOX group** – Cells were cultured in DMEM for 24 hours, followed by treatment with DOX (10 µM) at 37 °C for 24 hours.

3. **DOX + peonidin 50 group** – Cells were pre-treated with 50 μg/mL peonidin for 24 hours, followed by DOX (10 μM) treatment for another 24 hours.
4. **DOX + peonidin 100 group** – Cells were pre-treated with 100 μg/mL peonidin for 24 hours, followed by DOX (10 μM) treatment for another 24 hours.
5. **DOX + peonidin 150 group** – Cells were pre-treated with 150 μg/mL peonidin for 24 hours, followed by DOX (10 μM) treatment for another 24 hours.

Evaluation of cell viability & proliferation using MTT assay

The impact of treatment on proliferation was evaluated using an MTT assay, which was conducted at 24, 48, and 72 h post-treatment. The MTT reagent was added to the cells according to the manufacturer's protocol and incubated for 3–4 hours. This procedure allows the viable cells to reduce MTT into formazan crystals [9]. Then, the solubilization solution (such as DMSO) is added to solubilize the crystals, and its absorbance is measured at 570 nm through a microplate reader spectrophotometer. The inhibition of cell growth was calculated as a % of surviving treated cells compared to control cells using the formula:

$$\%\ Inhibition = 100 - \frac{Test\ absorbance - blank\ absorbance}{control\ absorbance - blank\ absorbance} \times 100$$

Lactate Dehydrogenase (LDH) Activity Assay

The treated cells were used to assess cellular injury by measuring lactate dehydrogenase (LDH) activity [10]. The culture medium from each well was collected and analysed for LDH activity using the LDH assay kit. Briefly, 100 μL of the culture medium was collected from each well and transferred to a 96-well plate. Then, 100 μL of LDH reaction mixture was added to each sample. The plate was gently mixed and incubated at room temperature for 30 min in the dark to allow the enzymatic reaction to occur in the reaction mixture. Then the absorbance of the solution was measured at 490 nm (for LDH activity) and 680 nm (for background correction) using a microplate reader Spectrophotometer. The final absorbance value was obtained by subtracting the absorbance at 490 nm from the absorbance at 680 nm. LDH release was calculated as a % using the formula:

$$LDH\ Release(\%) = \frac{Absorbance\ of\ treated\ sample}{Absorbance\ of\ maximum\ LDH\ release\ control} \times 100$$

Evaluation of ROS (Reactive Oxygen Species) Production:

The ROS production in H9c2 cells was evaluated using the DCF-DA fluorescent dye method. Initially, the H9c2 cells were seeded in culture plates and treated with three different concentrations of the sample, along with 150 μM DOX, for 8 hours to induce oxidative stress [11]. Then, DCF-DA (10 μM) solution was added to the cells, and the plates were incubated at 37°C for 30 min in a humidified 5% CO_2 atmosphere. This procedure allows intracellular uptake and conversion of DCF-DA into its fluorescent form.

Then, cells removed from DMEM medium were washed with pH 7.4 PBS (0.01 M) twice to eliminate any excess dye. Then, the cells were lysed using a 1% Triton X-100 solution in PBS and incubated at 37°C for 10 minutes. The fluorescence intensity that indicates ROS level was measured using a fluorescence microplate reader at 490 nm (excitation wavelength) and 525 nm (emission wavelength). The experiment was conducted in triplicate to ensure the accuracy and consistency of the results. The intensity of fluorescence directly corresponds to the amount of ROS present in the cells, which allows quantification of oxidative stress levels.

Evaluation of Lipid Peroxidation

The TBARS assay was employed to evaluate the lipid peroxidation of treated cells by measuring MDA levels [12]. The culture medium from H9c2 cells, seeded in 6-well plates for all treatments, was removed and transferred into 15 mL tubes. The attached cells were then removed by trypsinization, followed by centrifugation. The cells were trypsinized, transferred into 2 mL Eppendorf tubes, and diluted to approximately 1×10^7. Then, the cells were centrifuged and resuspended in PBS containing 1X BHT. Then, a 150 μL aliquot of sample supernatant was transferred into a 96-well black fluorescence plate, and the fluorescence was measured using a microplate reader at 540 nm (excitation wavelength) and 590 nm (emission wavelength). The MDA concentration was determined based on the standard curve that provides lipid peroxidation levels in the treated cells.

Evaluation of SOD Activity

The SOD (superoxide dismutase) activity was evaluated using a colorimetric assay kit as per the manufacturer's protocol [13]. The procedure was briefly described; the cells in the cell suspensions were lysed using 100 μL of 0.1 M Tris/HCl buffer (containing 0.5% Triton X-100, 5 mM β-ME, and 0.1 mg/mL of PMSF at pH 7.4). The lysed cells were then transferred into 2 ml Eppendorf tubes and centrifuged at 15,000 × g for 5 min at 4°C to separate the supernatant. Then it was centrifuged and 20 μL of the supernatant was pipetted into a 96-well assay plate. Then,

20 µL of enzyme solution and 200 µL of WST solution were added to each well, and the plates were incubated at 37°C for 20 minutes to allow the reaction to proceed. After completion of incubation, SOD activity was measured at 450 nm using a plate reader (Biotek ELX 800, Gen 5 software) by recording absorbance at 450 nm.

***In-silico* studies**:
An in-silico docking study was performed to evaluate the binding interactions and inhibitory activity of peonidin against significant biological targets involved in cardiovascular processes, such as ACE (Angiotensin-Converting Enzyme) and ECE-1 (Endothelin-Converting Enzyme-1) enzymes [14]. The target proteins play a significant role in regulating blood pressure and cardiovascular function. The docking stimulations were carried out using the iGEM-DOCK v2.1 software, which allows for the evaluation of the interaction of peonidin against these enzymes at the molecular level.

The Protein Data Bank (PDB) was utilized to collect the crystal structure of target proteins in this study. Before docking, the protein structures were prepared by removing all water molecules and co-crystallized ligands using Discovery Studio Visualizer. The ligands, peonidin and captopril, were energy-minimized and converted to the suitable format using Open Babel. Default grid sizes were utilized in iGEMDOCK with a docking region covering the active site of the enzyme. The docking was performed with a population size of 200, 70 generations, and two solutions per ligand.

This research utilizes captopril, a well-documented ACE inhibitor used in the management of hypertension and heart failure, as a reference standard. The findings obtained in this research provide essential information about the potential therapeutic uses of peonidin in cardiovascular therapies. The validation of the docking protocol was verified by conducting re-docking of co-crystallized ligands, and RMSD values were determined to ensure the reliability of the docking parameters.

RESULTS AND DISCUSSION

MTT assay was conducted to assess the viability of H9c2 cells treated with DOX and peonidin at different concentrations over 24, 48, and 72 hours. The control group exhibits nearly 100% cell viability at all time points, confirming the normal growth and proliferation of untreated cells. The cells treated with 150 µM DOX alone exhibit a time-dependent reduction in cell viability. The cell viability reduced to 68.58% at 24 hours, whereas 38.36% viability was observed at 48 hours, and further decreased to 24.69% at 72 hours, indicating the severe cytotoxic effects of DOX over time.

The therapy of DOX with various concentrations of peonidin shows a protective action against DOX-induced cytotoxicity. The cell viability of 50 µg/mL peonidin shows a comparatively low value of 68.19% at 24 hours and is enhanced to 62.05% at 48 hours. A viability of 80.46% was observed at 72 h, indicating the partial protection efficiency of peonidin at this dose. The increased dose of 100 µg/mL of peonidin results in a marked improvement, with 82.14% viability at 24 hours, 88.36% at 48 hours, and 90.34% at 72 hours. This indicates an enhanced cell survival efficiency of peonidin with increasing dose. At the highest dose of 150 µg/mL, peonidin almost restores cell viability to that of the controls, with 94.98% at 24 h, 98.98% at 48 h, and 99.74% (Table 1) at 72 h, indicating a significant cytoprotective effect against DOX-induced toxicity. These findings demonstrate that peonidin strongly counteracts DOX-induced cytotoxicity, providing strong protection and maintaining cell viability. The comparative results were shown in Figure 1, and the microscopic cell line observation was shown in Figure 2.

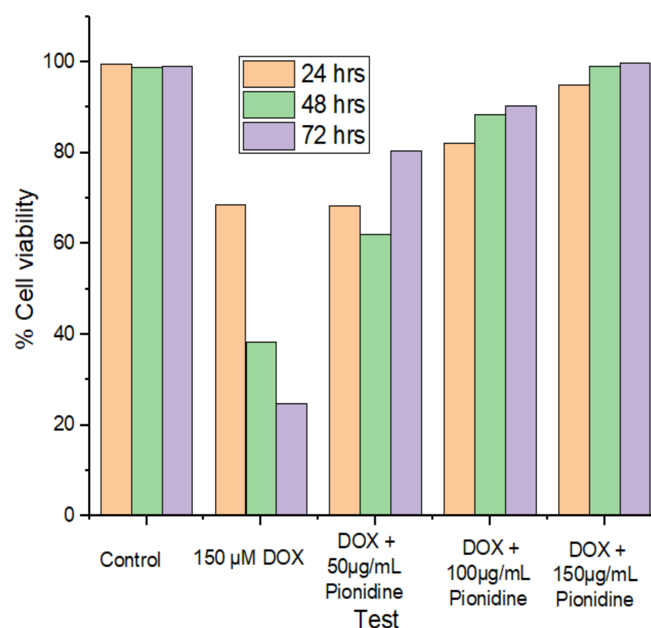


Figure 1: Effect of peonidin on Cell viability

The values are the means ± SEM(n=6) Doxorubicin administration significantly decreased the cell viability at different time intervals when compared to control.

Table 1: Cell viability results of peonidin

S.No	Test	% Cell viability observed at (Mean ± SEM)		
		24 hrs	48 hrs	72 hrs
1	Control	99.76 ± 0.158	100.02 ± 0.917	100.87 ± 0.781
2	DOX (150 µM)	68.58 ± 0.237	38.36 ± 0.552	24.69 ± 0.663
3	DOX + Peonidin 50 µg/mL	68.19 ± 0.591	62.05 ± 0.596	80.46 ± 0.520
4	DOX + Peonidin 100 µg/mL	82.14 ± 0.229	88.36 ± 0.417	90.34 ± 0.401
5	DOX + Peonidin 150 µg/mL	94.98 ± 0.467	98.98 ± 0.225	99.74 ± 0.636

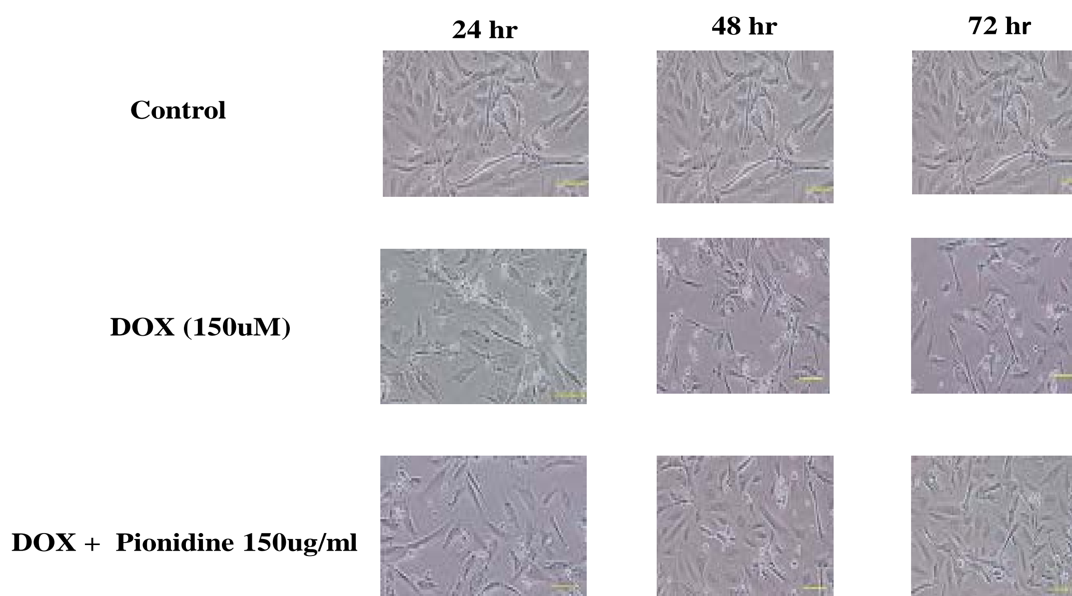


Figure 2: Cytotoxic effect of peonidin on H9c2 cells

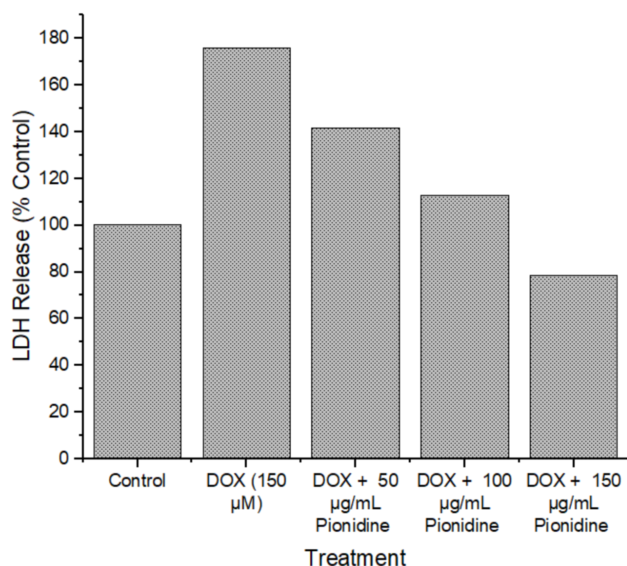
Effect of peonidin on LDH

The extent of cellular damage and loss of membrane integrity in H9c2 cells treated with DOX alone and in combination with peonidin at different concentrations was evaluated using the LDH assay. The results suggest that the control group exhibits a baseline LDH release of 100.24%, confirming minimal cell damage under normal conditions. The cells treated with 150 µM DOX alone result in a substantial increase in LDH release to 175.84% indicating severe DOX-induced cytotoxicity and membrane damage. The treatment of peonidin at 50 µg/mL slightly reduced LDH release to 141.71% demonstrating a mild protective effect of peonidin at low concentration. The increase of peonidin dose to 100 µg/mL results in an enhanced protective effect of 112.73% suggesting that peonidin effectively alleviates DOX-induced membrane damage. At a dose of 150 µg/mL peonidin, LDH release decreased drastically to 78.40%, implying that peonidin exhibits a strong protective effect against DOX-induced cytotoxicity at higher concentrations. These findings suggest that peonidin mitigates DOX-induced cellular injury by reducing LDH release in a dose-dependent manner. Figure 3A gives the comparative LDH assay study results noticed in this study.

Effect of Peonidin on ROS release

The effect of peonidin on ROS release in H9c2 cells treated with DOX alone and in combination with peonidin at different concentrations was evaluated. Initially, the cells treated with DOX showed a significant elevation in ROS levels, reaching 191.13% compared to the control (99.85%), indicating that DOX induces severe oxidative stress. The involvement of peonidin at 50 µg/mL reduces ROS release to 164.82% demonstrating a partial protective effect. The increase in the peonidin concentration to 100 µg/mL further decreases ROS levels to 95.13% suggesting the effective restoration of oxidative balance in cell lines. The rise in peonidin concentration to 150 µg/mL exhibits a strong antioxidant effect. At this concentration, the ROS release is drastically reduced to 61.29% suggesting a dose-dependent protective role against DOX-induced oxidative stress

in H9c2 cells. These findings indicate that peonidin effectively mitigates oxidative damage and enhances cellular defense mechanisms. The comparative results in this study were presented in Figure 3B.



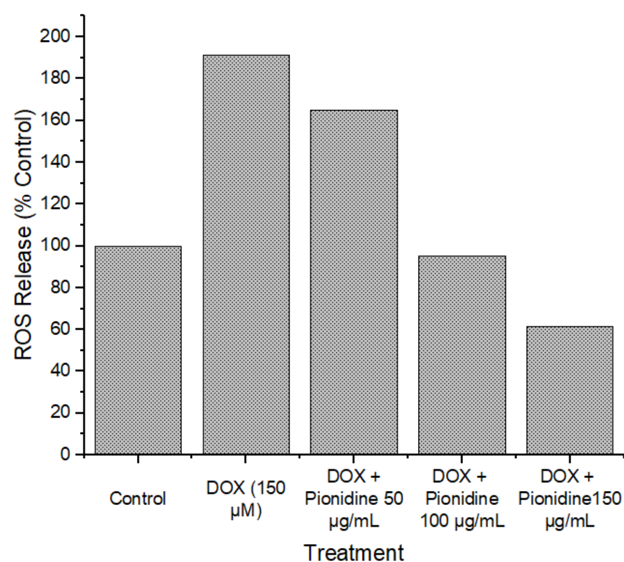


Figure 3: Effect of peonidin on LDH (A) release and ROS (B) release in the H9c2 cells treated with DOX

Evaluation of Lipid Peroxidation:

The impact of peonidin on lipid peroxidation was analyzed by determining the MDA level in DOX-treated H9c2 cells and in combination with varying concentrations of peonidin. DOX treatment significantly increased MDA levels (85.58±0.134 AU), indicating that DOX induces widespread lipid peroxidation and oxidative damage to membranes compared to controls (41.58±0.134 AU). The Co-treatment with 50 µg/mL peonidin causes a minor decrease in MDA content (81.57±0.365 AU), indicating a weak protective action against DOX-induced lipid peroxidation. Yet, the more substantial decrease with 100 µg/mL peonidin (52.39±0.226 AU) exhibits a strong antioxidant activity in counteracting DOX-induced oxidative stress.

Remarkably, at 150 µg/mL concentration of peonidin, it exhibits remarkable lipid peroxidation activity (36.17±0.213 AU). The results of these comparisons, as shown in Figure 4, demonstrate that peonidin significantly suppresses DOX-caused lipid peroxidation dose-dependently by neutralizing ROS and stabilizing the cellular membrane, thereby preventing oxidative injury in H9c2 cells.

Evaluation of SOD Activity:

The impact of peonidin on SOD in DOX-only-treated and DOX-plus-varying concentrations of peonidin -treated H9c2 cells was assessed. SOD activity was found to be 99.36 ± 0.251 AU in the control group, which is a normal level of enzymatic antioxidant defense. However, treatment with DOX drastically reduced SOD activity to 36.59 ± 0.306 AU, suggesting significant oxidative stress and impaired antioxidant enzyme function due to the effect of DOX treatment.

The co-treatment with peonidin at 50 µg/mL results in a slight increase in SOD activity (41.03 ± 0.119 AU), suggesting a mild protective effect at a low concentration of peonidin. The increase in the peonidin treatment concentration to 100 µg/mL displays a more substantial restoration of SOD activity (73.24 ± 0.257 AU), demonstrating improved antioxidant defense.

The further increase of treatment concentration to 150 µg/mL restores SOD activity to 97.85 ± 0.313 AU and is noticed to be very close to control levels, suggesting that peonidin significantly counteracts DOX-induced oxidative stress by enhancing endogenous antioxidant defenses. The comparative representation of various treatments on SOD activity is shown in Figure 4B.

IN SILICO STUDIES

The cardioprotective activity of peonidin was further confirmed by performing docking studies with ACE and ECE-1 enzymes, which are the key enzymes involved in cardiovascular diseases. The molecular docking study results of peonidin against the

ACE enzyme demonstrate significant differences in binding affinity and interaction energies compared to the reference standard, captopril. Peonidin exhibits a stronger binding affinity, with a total energy of -95.7223 kcal/mol, compared to captopril (83.6409 kcal/mol), indicating a more stable interaction with ACE. The van der Waals (VDW) interactions of peonidin were higher (-65.3167 kcal/mol) than captopril (-55.5514 kcal/mol), suggesting better surface complimentarily within the ACE active site. Whereas the hydrogen bonding contributions were also observed to be higher for peonidin (-30.4056 kcal/mol) than for captopril (-21.9098 kcal/mol), this reinforces its strong binding potential. However, captopril exhibits electrostatic interactions (-6.1798 kcal/mol). In contrast, peonidin had none (0 kcal/mol), indicating that peonidin's binding to ACE is predominantly governed by van der Waals and hydrogen bonding interactions rather than ionic forces. The average contact pair values were 22.6818 for peonidin and 27.1429 for captopril, suggesting slightly fewer but stronger interactions for peonidin.

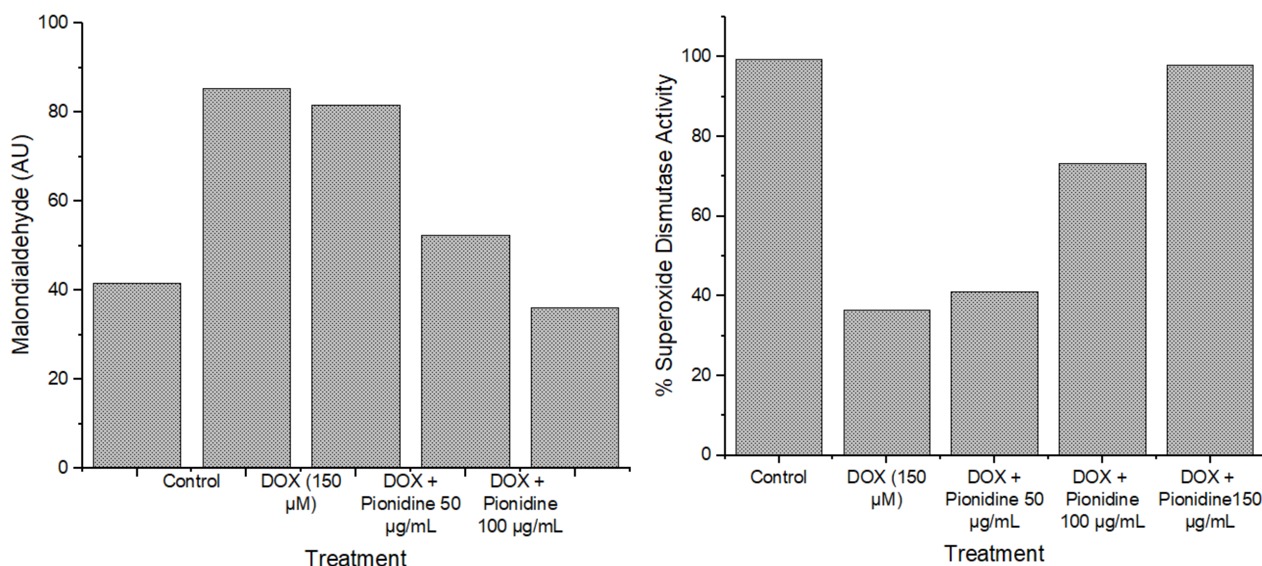


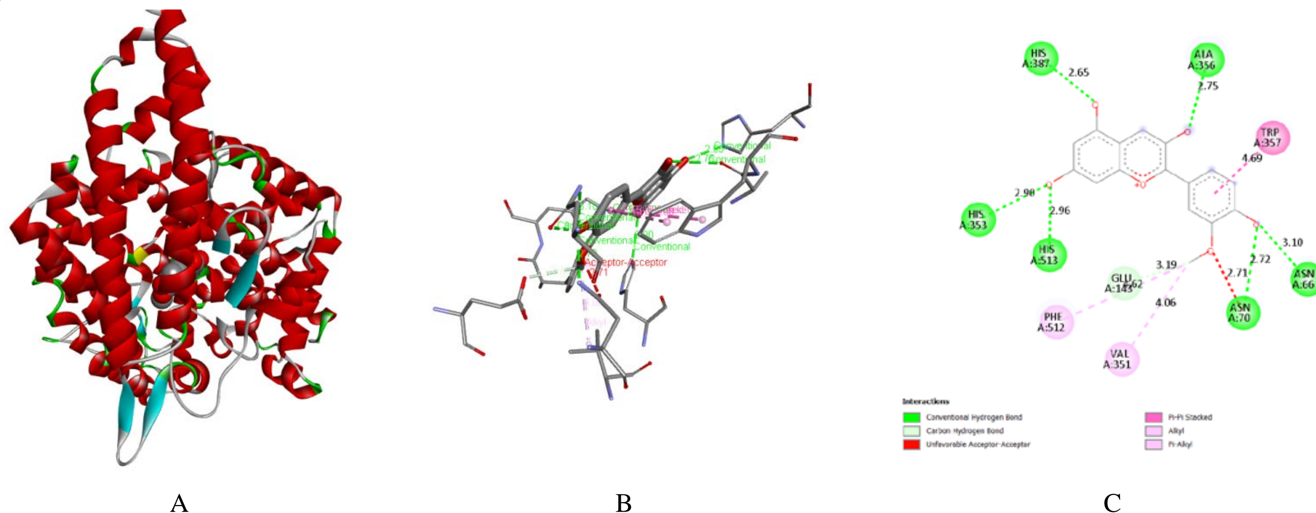
Figure 4: Lipid peroxidation (A) and SOD Activity (B) results observed in the study

The re-docking of the co-crystallized ligand (captopril) into the ACE binding pocket was performed to evaluate the docking efficiency results. The RMSD value between the docked & crystallographic conformations was 1.43 Å, indicating the reliability and precision of the docking protocol. These results highlight that peonidin has a superior binding affinity, suggesting its potential as a natural ACE inhibitor and making it a promising candidate for further investigation in hypertension management. Figure 5 presents the representative docking images of peonidin with ACE.

The docking analysis of peonidin with ECE-1 demonstrates a significantly stronger binding affinity with a total energy of -78.0863 kcal/mol. The reference standard compound captopril exhibits a lower total energy of -50.894 kcal/mol, indicating that peonidin exhibits a more stable interaction with ECE-1. The more favourable VDW peonidin (-61.3309 kcal/mol) interactions than captopril (-44.2188 kcal/mol) indicate more stable hydrophobic and steric interactions in the active site. Peonidin also provides more stable hydrogen bonds (-16.7554 kcal/mol) than captopril (-6.67522 kcal/mol), which improves the stability and selectivity of the compound toward ECE-1. Notably, both compounds don't exhibit electrostatic interactions (0 kcal/mol), indicating that VDW and hydrogen bonding forces primarily drove their binding.

Similar average contact pair values were observed for peonidin (20.0909) and captopril (20.8571), suggesting comparable interaction efficiencies for both compounds. The docking methodology for ECE-1 was validated by performing a re-docking study of the co-crystallized ligand (captopril) into the ECE-1 binding pocket. The RMSD value between the docked and crystallographic conformations was found to be 1.67 Å, confirming the reliability & precision of the docking protocol used in this study. These results show greater binding affinity of peonidin to ECE-1, establishing its potential as a natural inhibitor of ECE-1. Figure 6 depicts the representative docking images of peonidin with ECE-1. The docking study results indicate that peonidin is a promising natural compound for further exploration as an inhibitor of ACE and ECE-1,

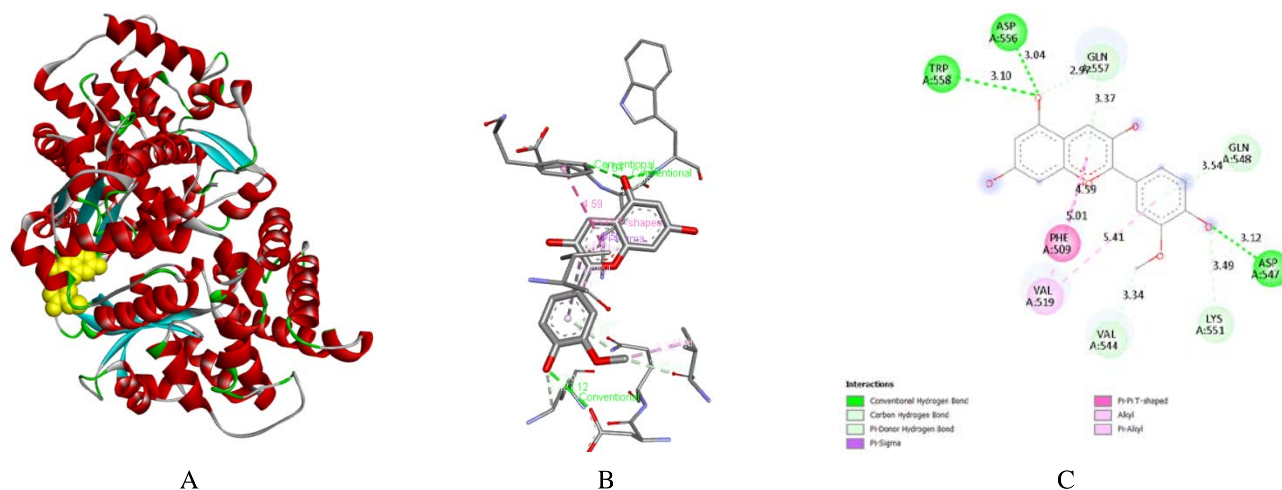
potentially offering a therapeutic strategy for diseases such as hypertension and cardiovascular diseases.



A B C

Docked ligand (A); 3D (B) 2D images of showing peonidin binding with ACE

Figure 5: Representative molecular docking results of peonidin with ACE



A B C

Docked ligand (A); 3D (B) 2D images of showing peonidin binding with ECE-1

Figure 6: Representative molecular docking results of peonidin with ECE-1

CONCLUSION

The purpose of this research was to explore the cardioprotective potential of peonidin against doxorubicin (DOX)- induced cytotoxicity in H9c2 cells by assessing its protective role on cell viability, oxidative stress, lipid peroxidation, and antioxidant defense mechanisms. Additionally, an in-silico molecular docking analysis was employed to determine the binding affinity of peonidin with the relevant cardiovascular enzymes, including ACE and ECE-1. The findings in this research reveal that DOX exposure to H9c2 cells significantly decreases cell survival in a time-dependent manner, with severe cytotoxic effects. However, co-exposure to peonidin exhibits a dose-dependent protective effect. The outcome of the MTT assay confirms that peonidin enhances cellular viability in a dose-dependent manner, cancelling the toxicity caused by DOX. In addition, the LDH assay indicates that peonidin significantly inhibits membrane injury, whereas the ROS and lipid peroxidation assays indicate its significant antioxidant activity, substantially reducing markers of oxidative stress. The recovery of SOD activity also demonstrates its ability to enhance cellular antioxidant defense mechanisms. The molecular docking analysis supported the experimental results by indicating that peonidin has a higher binding affinity towards both ACE and ECE-1 than captopril. Docking results show that peonidin is more stable in binding

with ACE (-95.7223 kcal/mol) and ECE-1 (-78.0863 kcal/mol) than captopril (-83.6409 kcal/mol for ACE and -50.894 kcal/mol for ECE-1). The stronger van der Waals and hydrogen bonding interactions of peonidin also position it as a better natural inhibitor of these enzymes, which are of significant importance in cardiovascular disease. The merits of this study lie in its comprehensive evaluation of peonidin's protective effect at both cellular and molecular levels. The experimental results, in combination with in silico results, provide strong evidence for peonidin's therapeutic potential against doxorubicin (DOX)-induced cardiotoxicity. Furthermore, the plant origin of peonidins, with their superior efficacy, makes them an excellent lead for designing safer cardioprotective drugs. Subsequent in vivo studies are needed to evaluate the cardiovascular protective ability of peonidin.

FINANCIAL ASSISTANCE
NIL

CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTION
Jyothirmai Namathoti and Rajeshwari Pasupula designed the concept. Jyothirmai Namathoti performed the experimental work and contributed to the preparation of the manuscript. Rajeshwari Pasupula supervised this research and contributed to interpreting statistical data.

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