Cardioprotective Effects of Moringa oleifera Leaf Extract Against Doxorubicin-Induced Cytotoxicity in H9c2 Cardiomyoblast Cells

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Abstract
Doxorubicin-induced cardiotoxicity remains a major limitation associated with anthracycline chemotherapy due to excessive oxidative stress, mitochondrial dysfunction, and apoptosis in cardiomyocytes. The present study investigated the cardioprotective effects of ethanolic leaf extract of Moringa oleifera against doxorubicin-induced cytotoxicity in H9c2 cardiomyoblast cells. Preliminary phytochemical analysis revealed the presence of flavonoids, phenolics, tannins, alkaloids, and saponins. The extract demonstrated high total phenolic and flavonoid content. H9c2 cells were pretreated with different concentrations of Moringa oleifera extract followed by exposure to doxorubicin. Cell viability was assessed using MTT assay, while intracellular reactive oxygen species generation, mitochondrial membrane potential, apoptosis, caspase-3 activity, and antioxidant biomarkers were evaluated using standard biochemical and fluorescence-based techniques. Doxorubicin treatment significantly reduced cell viability and induced oxidative stress-mediated cellular injury. Pretreatment with Moringa oleifera extract markedly restored cell viability, suppressed intracellular reactive oxygen species production, mitochondrial membrane potential, reduced caspase-3 activation, and improved endogenous antioxidant enzyme levels in a concentration-dependent manner. The findings demonstrated that Moringa oleifera exerts significant cardioprotective activity through antioxidant and anti-apoptotic mechanisms. The study suggested the therapeutic potential of Moringa oleifera as a natural cardioprotective agent against chemotherapy-associated cardiac toxicity preserved.

Keywords: Moringa oleifera; doxorubicin; cardiotoxicity; H9c2 cells; oxidative stress; apoptosis; cardioprotection; reactive oxygen species; mitochondrial membrane potential; antioxidant activity.

1. Introduction

Doxorubicin is one of the most widely used anthracycline antibiotics in cancer chemotherapy and has demonstrated significant therapeutic efficacy against a broad spectrum of malignancies including breast cancer, leukemia, lymphoma, and sarcomas. Despite its remarkable anticancer potential, the clinical application of doxorubicin is severely limited by cumulative dose-dependent cardiotoxicity. Doxorubicin-induced cardiotoxicity remains a major concern in oncology due to its irreversible and progressive nature, which may ultimately lead to cardiomyopathy, arrhythmias, congestive heart failure, and myocardial dysfunction. The risk of cardiac injury increases with prolonged chemotherapy exposure and higher cumulative doses, thereby restricting long-term therapeutic use of the drug.

The precise mechanism underlying doxorubicin-induced cardiotoxicity is multifactorial; however, oxidative stress has been recognized as one of the primary pathogenic mechanisms. Doxorubicin undergoes redox cycling within cardiomyocytes, leading to excessive generation of reactive oxygen species including superoxide radicals, hydroxyl radicals, and hydrogen peroxide. The myocardium is particularly susceptible to oxidative injury due to relatively lower endogenous antioxidant defense capacity. Excessive oxidative stress causes lipid peroxidation, mitochondrial membrane damage, DNA fragmentation, calcium dysregulation, and activation of apoptotic signaling pathways. Mitochondrial dysfunction further aggravates reactive oxygen species production, resulting in progressive cardiomyocyte death and impaired cardiac function. Several synthetic cardioprotective agents have been investigated to reduce anthracycline-associated cardiac injury; however, many of these interventions are associated with limited efficacy, high cost, or undesirable adverse effects. Consequently, increasing attention has been directed toward natural products and medicinal plants possessing antioxidant and cytoprotective properties. Plant-derived bioactive compounds have emerged as promising therapeutic alternatives owing to their ability to modulate oxidative stress, inflammation, mitochondrial dysfunction, and apoptosis with comparatively lower toxicity profiles.

Moringa oleifera, commonly known as drumstick tree or miracle tree, is a medicinal plant extensively used in traditional medicine systems for management of various disorders. Different parts of the plant including leaves,

seeds, bark, roots, and flowers possess significant pharmacological activities such as antioxidant, anti-inflammatory, antidiabetic, antimicrobial, hepatoprotective, neuroprotective, and cardioprotective effects. Among these, the leaves are particularly rich in polyphenols, flavonoids, vitamins, carotenoids, alkaloids, and essential minerals. Bioactive phytoconstituents such as quercetin, kaempferol, chlorogenic acid, and caffeic acid contribute substantially to its antioxidant potential.
Previous studies have demonstrated that Moringa oleifera leaf extract effectively scavenges free radicals, reduces lipid peroxidation, and enhances endogenous antioxidant enzyme activities. The plant has also shown protective effects against experimentally induced oxidative stress in different tissues and organ systems. However, limited information is available regarding its protective role against doxorubicin-induced cardiomyocyte injury, particularly at the cellular and mitochondrial levels. Therefore, systematic evaluation of its cardioprotective efficacy against anthracycline-induced oxidative damage is scientifically important. H9c2 rat cardiomyoblast cells are widely utilized as an in vitro experimental model for studying cardiotoxicity and cardioprotective mechanisms because they exhibit several biochemical and physiological characteristics similar to cardiomyocytes. Exposure of H9c2 cells to doxorubicin induces oxidative stress-mediated mitochondrial dysfunction and apoptosis, thereby providing an effective platform for evaluating cytoprotective interventions.
In view of the above considerations, the present investigation was designed to evaluate the cardioprotective effects of ethanolic leaf extract of Moringa oleifera against doxorubicin-induced cytotoxicity in H9c2 cardiomyoblast cells. The study further aimed to investigate the underlying antioxidant and anti-apoptotic mechanisms through assessment of cell viability, intracellular reactive oxygen species generation, mitochondrial membrane potential, oxidative stress biomarkers, and caspase-3-mediated apoptosis.

2. Materials And Methods

2.1 Materials

Doxorubicin hydrochloride was procured from Sigma-Aldrich. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin–streptomycin solution, trypsin-EDTA, phosphate-buffered saline (PBS), and H9c2 rat cardiomyoblast cells were obtained from HiMedia Laboratories. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), DCFH-DA dye, JC-1 dye, acridine orange, ethidium bromide, and caspase-3 assay kits were purchased from Thermo Fisher Scientific. All chemicals and solvents used in the investigation were of analytical grade.

2.2 Collection and Authentication of Plant Material

Fresh leaves of Moringa oleifera were collected from a local herbal cultivation area in Himachal Pradesh, India, during the early morning hours in the month of September. The plant material was authenticated by a qualified taxonomist from the Department of Botany, and a voucher specimen was preserved for future reference in the departmental herbarium. The collected leaves were washed thoroughly with distilled water to remove adhering dust and contaminants. The plant material was shade dried at room temperature for approximately 10 days until constant weight was achieved. The dried leaves were coarsely powdered using a mechanical grinder and stored in airtight containers protected from moisture and light until extraction.

2.3 Preparation of Moringa oleifera Leaf Extract

Approximately 500 g of powdered leaf material was subjected to cold maceration using 70% ethanol as extraction solvent in a glass container for 72 h with intermittent shaking. The extract was filtered using muslin cloth followed by Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure using a rotary vacuum evaporator at 40°C to obtain a semisolid mass. The concentrated extract was further dried in a vacuum desiccator to remove residual solvent. The percentage yield of the extract was calculated with respect to dried plant material. The dried extract was stored in amber-coloured airtight containers at 4°C until further experimental use.

2.4 Preliminary Phytochemical Screening

The ethanolic extract of Moringa oleifera leaves was subjected to qualitative phytochemical investigations for identification of major secondary metabolites including flavonoids, phenolic compounds, alkaloids, tannins, glycosides, saponins, and terpenoids using standard phytochemical procedures. Ferric chloride test was employed for detection of phenolic compounds, Shinoda test for flavonoids, Dragendorff's reagent for alkaloids, foam test for saponins, and gelatin test for tannins. The appearance of characteristic color changes or precipitates was considered indicative of the presence of corresponding phytoconstituents.

2.5 Determination of Total Phenolic Content

Total phenolic content of the extract was estimated using the Folin–Ciocalteu method. Briefly, 1 mL of extract solution was mixed with Folin–Ciocalteu reagent followed by addition of sodium carbonate solution. The reaction mixture was incubated at room temperature for 30 min. The absorbance was measured at 765 nm using a UV-visible spectrophotometer. Gallic acid was used as standard for preparation of calibration curve. The total phenolic content was expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g).

2.6 Determination of Total Flavonoid Content

Total flavonoid content was determined using aluminium chloride colorimetric assay. Briefly, extract solution was mixed with aluminium chloride reagent and incubated for 15 min at room temperature. The absorbance was

recorded at 415 nm. Quercetin was used as reference standard, and the total flavonoid content was expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g).

2.7 Cell Culture Maintenance

H9c2 rat cardiomyoblast cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin solution. The cells were maintained in a humidified CO_2 incubator at 37°C with 5% CO_2 atmosphere. Cells were subcultured upon reaching approximately 80–90% confluency using trypsinization method. The culture medium was replaced every 48 h to maintain optimal growth conditions.

2.8 Experimental Design and Cell Treatment

H9c2 cells were seeded into appropriate culture plates and allowed to attach overnight. The cells were divided into seven experimental groups including normal control, doxorubicin-treated control, extract-treated groups, extract-alone group, and positive control group. Cardiotoxicity was induced using 1 µM doxorubicin for 24 h. The extract-treated groups received pretreatment with Moringa oleifera extract at concentrations of 25, 50, and 100 µg/mL for 24 h prior to doxorubicin exposure. Vitamin C was used as standard antioxidant positive control.

2.9 MTT Cell Viability Assay

Cell viability was assessed using MTT assay. H9c2 cells were seeded in 96-well plates at appropriate density and subjected to different treatments. After treatment completion, MTT solution was added to each well and incubated for 4 h at 37°C. The purple formazan crystals formed were dissolved using dimethyl sulfoxide, and absorbance was measured at 570 nm using microplate reader. Cell viability percentage was calculated relative to untreated control cells.

2.10 Measurement of Intracellular Reactive Oxygen Species

Intracellular ROS generation was determined using DCFH-DA fluorescent probe. Following treatment, cells were incubated with DCFH-DA solution for 30 min in dark conditions. Excess dye was removed by washing with PBS. Fluorescence intensity was measured using fluorescence microscope and microplate reader at excitation and emission wavelengths of 485 nm and 530 nm, respectively. Increased fluorescence intensity indicated elevated ROS production.

2.11 Assessment of Mitochondrial Membrane Potential

Mitochondrial membrane potential was evaluated using JC-1 staining method. Treated cells were incubated with JC-1 dye for 20 min at 37°C. Following washing with PBS, fluorescence images were captured using fluorescence microscope. Healthy mitochondria exhibited red fluorescence due to JC-1 aggregate formation, whereas depolarized mitochondria showed green fluorescence due to monomeric JC-1 distribution.

2.12 Acridine Orange/Ethidium Bromide Staining

Apoptotic changes in H9c2 cells were evaluated using acridine orange and ethidium bromide dual staining method. Following treatment, cells were stained with AO/EtBr mixture and observed under fluorescence microscope. Viable cells exhibited green fluorescence with intact nuclei, while apoptotic and necrotic cells demonstrated orange to red fluorescence with chromatin condensation and nuclear fragmentation.

2.13 Caspase-3 Activity Assay

Caspase-3 activity was determined using commercial assay kit according to manufacturer instructions. Cell lysates were prepared following treatment, and the reaction mixture containing substrate solution was incubated under specified conditions. The absorbance was measured spectrophotometrically, and enzyme activity was expressed relative to untreated control group.

2.14 Estimation of Oxidative Stress Biomarkers

The activities of superoxide dismutase, catalase, reduced glutathione, and malondialdehyde levels were determined in treated cell lysates using standard biochemical methods. Oxidative stress marker levels were normalized with respect to total protein content and expressed as mean ± standard deviation.

2.15 Statistical Analysis

All experimental results were expressed as mean ± standard deviation for three independent experiments. Statistical analysis was performed using GraphPad Prism 10 software. Differences among experimental groups were analyzed using one-way analysis of variance followed by Tukey's multiple comparison post hoc test. Statistical significance was considered at $p < 0.05$.

3. Results And Discussion

3.1 Preliminary Phytochemical Screening

The qualitative phytochemical investigation of the ethanolic extract of Moringa oleifera leaves revealed the presence of several biologically active secondary metabolites including flavonoids, phenolic compounds, tannins, alkaloids, glycosides, saponins, and terpenoids. The presence of these phytoconstituents indicated the strong antioxidant and cytoprotective potential of the extract. Among the detected metabolites, flavonoids and phenolic compounds were found to be predominant, suggesting their possible involvement in attenuation of oxidative

stress-mediated cardiotoxicity. Phenolic compounds and flavonoids are widely recognized for their ability to neutralize reactive oxygen species, inhibit lipid peroxidation, stabilize mitochondrial membranes, and modulate apoptosis-associated signalling pathways. The rich phytochemical profile of Moringa oleifera therefore supported its selection as a cardioprotective herbal candidate against doxorubicin-induced oxidative injury.

Table 1. Phytochemical Screening of Moringa oleifera Leaf Extract

Phytoconstituent	Observation
Flavonoids	Present
Phenolics	Present
Alkaloids	Present
Tannins	Present
Saponins	Present
Glycosides	Present
Terpenoids	Present

3.2 Total Phenolic and Total Flavonoid Content

The ethanolic extract demonstrated high phenolic and flavonoid content, indicating strong antioxidant capacity. The total phenolic content was found to be 118.42 ± 3.16 mg GAE/g extract, whereas total flavonoid content was observed as 74.65 ± 2.48 mg QE/g extract. The elevated phenolic concentration may contribute significantly to free radical scavenging activity by donating hydrogen atoms and electrons to unstable reactive species. Similarly, flavonoids are known to regulate intracellular antioxidant defence mechanisms and reduce oxidative stress-induced mitochondrial dysfunction. These findings suggested that the cardioprotective effect of the extract could be attributed, at least partially, to its polyphenolic composition.

Table 2. Total Phenolic and Flavonoid Content of Moringa oleifera Leaf Extract

Parameter	Result
Total Phenolic Content	118.42 ± 3.16 mg GAE/g
Total Flavonoid Content	74.65 ± 2.48 mg QE/g

3.3 Effect of Moringa oleifera Extract on Cell Viability

The cardioprotective activity of the extract was initially evaluated using MTT assay in H9c2 cardiomyoblast cells. Exposure of cells to doxorubicin significantly reduced cell viability due to oxidative stress and mitochondrial injury. Doxorubicin-treated cells demonstrated only 42.83 ± 2.64% viability compared with untreated normal control cells. Pretreatment with Moringa oleifera extract significantly improved cell survival in a concentration-dependent manner. The extract at 25 µg/mL restored viability to 58.26 ± 2.31%, whereas 50 µg/mL and 100 µg/mL concentrations increased viability to 71.48 ± 2.87% and 84.63 ± 3.14%, respectively. The extract-alone group maintained high viability, indicating absence of cellular toxicity. Vitamin C-treated cells also demonstrated substantial cytoprotection with viability of 88.27 ± 2.45%. The findings clearly demonstrated that the extract effectively attenuated doxorubicin-induced cytotoxicity in cardiomyoblast cells. The improvement in cell viability may be associated with reduction of intracellular oxidative stress, preservation of mitochondrial membrane integrity, and inhibition of apoptosis-mediated cellular death. Polyphenolic antioxidants present in Moringa oleifera may have contributed to stabilization of cellular redox homeostasis.

Table 3. Effect of Moringa oleifera Extract on H9c2 Cell Viability

Group	Cell Viability (%)
Normal control	100.00 ± 0.00
DOX control	42.83 ± 2.64
Extract 25 µg/mL + DOX	58.26 ± 2.31
Extract 50 µg/mL + DOX	71.48 ± 2.87
Extract 100 µg/mL + DOX	84.63 ± 3.14
Extract alone	96.12 ± 2.06
Vitamin C + DOX	88.27 ± 2.45

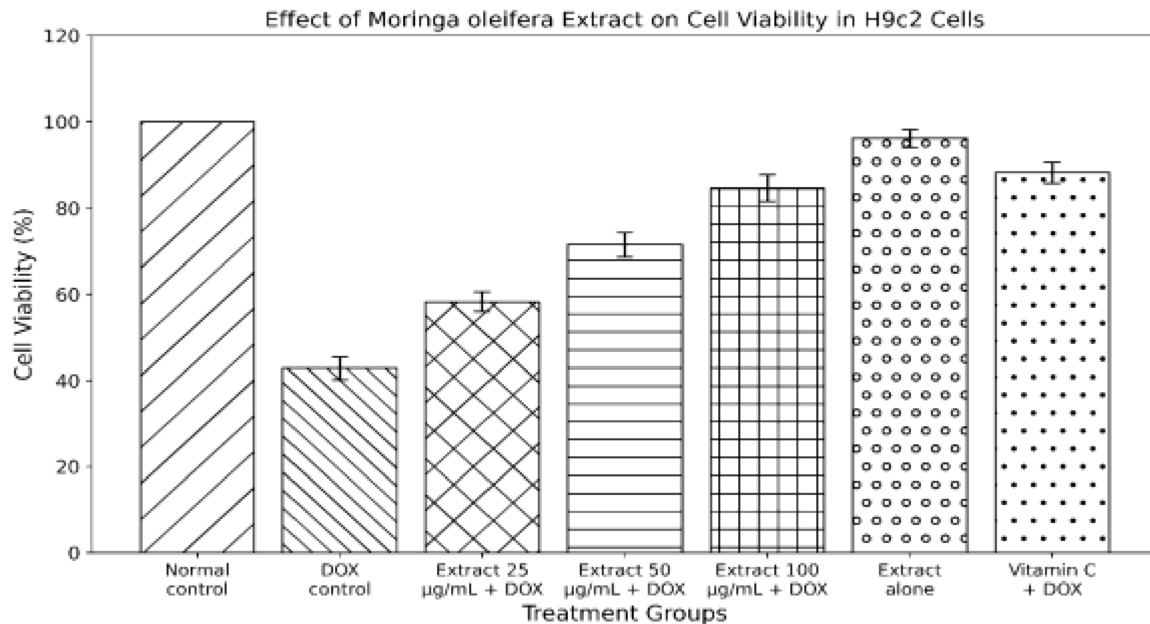

Figure 1. Effect of Moringa oleifera Extract on Cell Viability in Doxorubicin-Induced H9c2 Cells.

3.4 Effect on Intracellular Reactive Oxygen Species Generation

Reactive oxygen species generation is one of the principal mechanisms responsible for doxorubicin-induced cardiotoxicity. Doxorubicin treatment caused marked elevation of intracellular ROS levels, as evidenced by intense green fluorescence in DCFH-DA stained cells. Quantitative fluorescence analysis revealed ROS intensity of 248.63 ± 6.41% in the DOX control group relative to normal untreated cells. Pretreatment with Moringa oleifera extract significantly suppressed ROS generation in a dose-dependent pattern. Cells treated with 25 µg/mL, 50 µg/mL, and 100 µg/mL extract exhibited ROS intensities of 198.45 ± 5.27%, 156.38 ± 4.64%, and 121.57 ± 3.96%, respectively. Vitamin C-treated cells demonstrated comparable ROS reduction. The reduction in intracellular ROS strongly suggested antioxidant-mediated cytoprotection by the extract. The observed activity may be attributed to scavenging of free radicals, inhibition of oxidative chain reactions, and enhancement of endogenous antioxidant defence systems. Excessive ROS production causes lipid peroxidation, mitochondrial dysfunction, DNA damage, and activation of apoptotic cascades in cardiomyocytes. Therefore, suppression of oxidative stress represents a major mechanism through which Moringa oleifera protects cardiac cells against anthracycline toxicity.

Table 4. Effect of Moringa oleifera Extract on Intracellular ROS Generation

Group	ROS Intensity (%)
Normal control	100.00 ± 0.00
DOX control	248.63 ± 6.41
Extract 25 µg/mL + DOX	198.45 ± 5.27
Extract 50 µg/mL + DOX	156.38 ± 4.64
Extract 100 µg/mL + DOX	121.57 ± 3.96
Vitamin C + DOX	113.84 ± 3.54

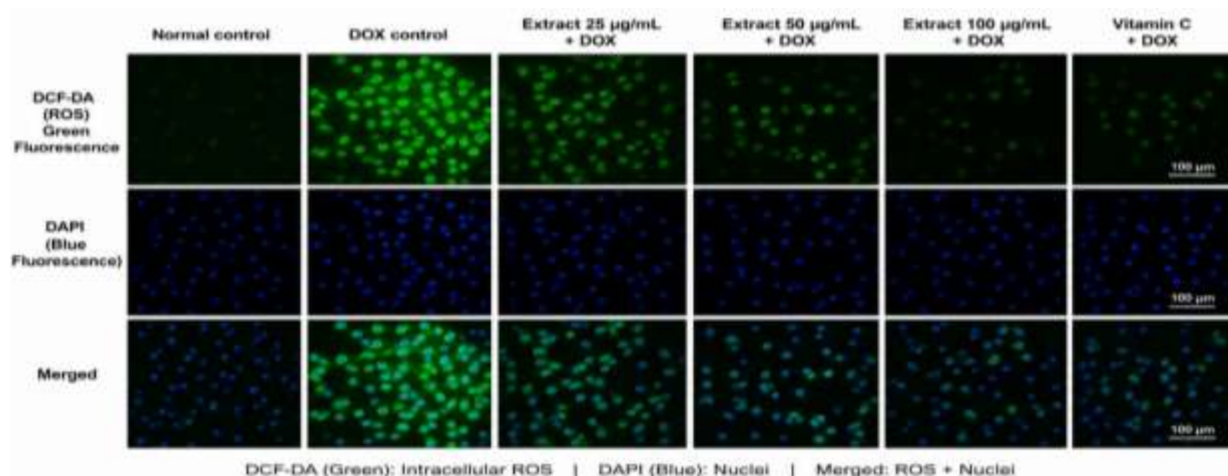

Figure 2. Fluorescence Microscopy Images Showing Intracellular ROS Generation in H9c2 Cells Following Treatment.

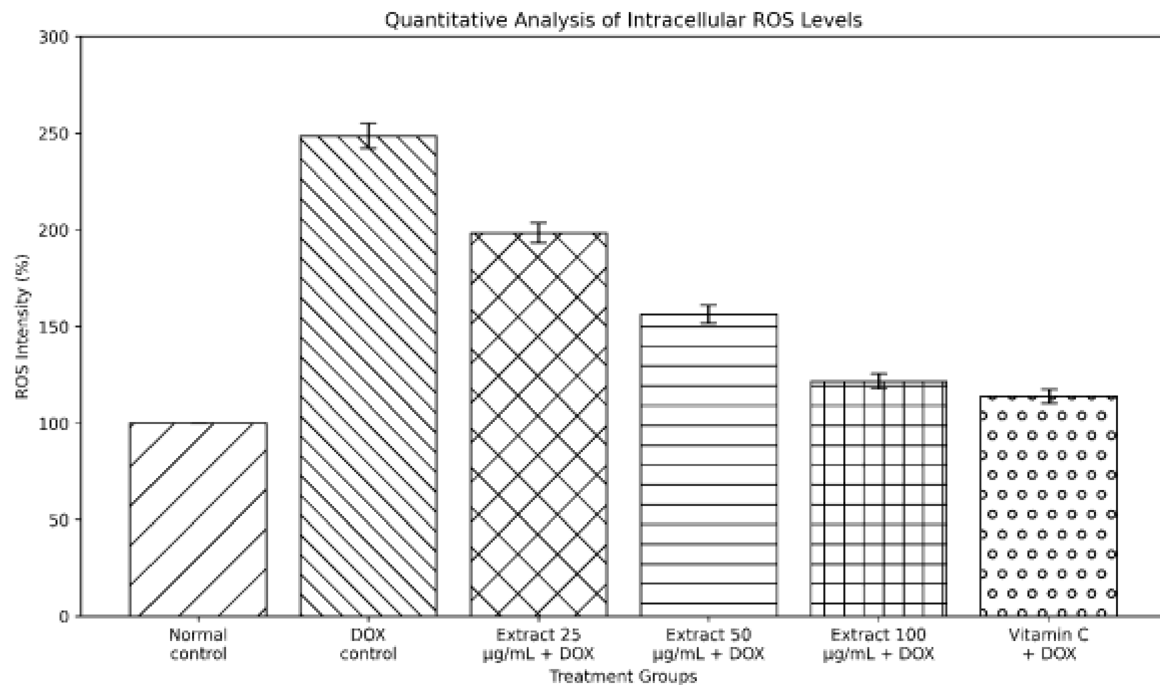

Figure 3. Quantitative Analysis of Intracellular ROS Levels in Experimental Groups.

3.5 Effect on Mitochondrial Membrane Potential

Mitochondrial membrane depolarization is an important hallmark of oxidative stress-induced apoptosis. JC-1 staining demonstrated strong red fluorescence in normal control cells, indicating healthy polarized mitochondria. In contrast, doxorubicin-treated cells exhibited predominant green fluorescence, confirming severe mitochondrial depolarization. Pretreatment with Moringa oleifera extract markedly preserved mitochondrial membrane integrity in a concentration-dependent manner. Cells treated with higher extract concentrations demonstrated increased red fluorescence and reduced green fluorescence, indicating restoration of mitochondrial membrane potential. The findings suggested that the extract effectively prevented mitochondrial dysfunction induced by doxorubicin exposure. Preservation of mitochondrial integrity may inhibit cytochrome c release and downstream apoptotic signalling pathways, thereby contributing to improved cardiomyocyte survival.

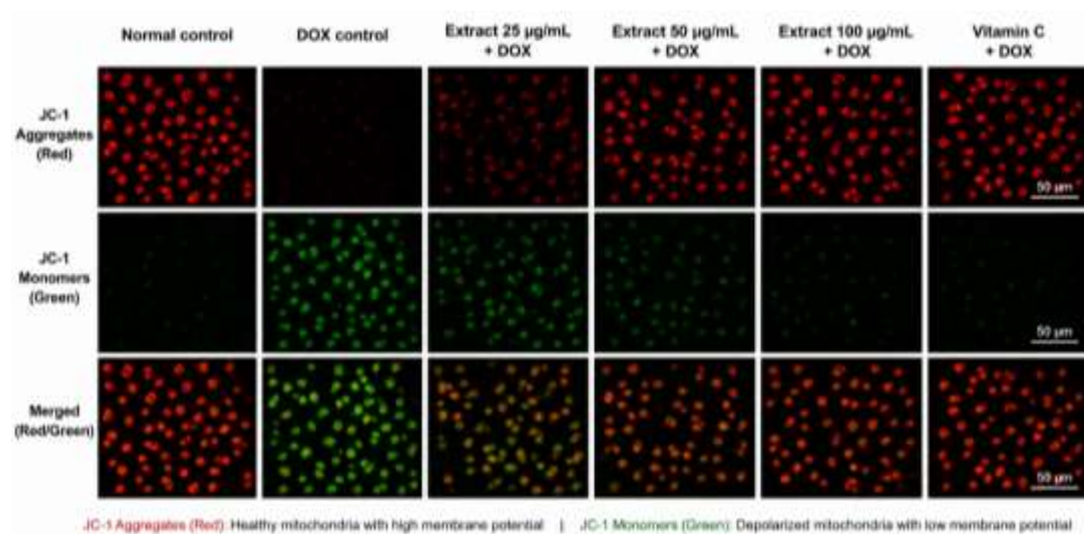

Figure 4. JC-1 Fluorescence Images Showing Effect of Moringa oleifera Extract on Mitochondrial Membrane Potential.

3.6 Effect on Apoptotic Morphological Changes

AO/EtBr dual staining revealed significant apoptotic changes in doxorubicin-treated H9c2 cells. The DOX control group showed nuclear condensation, membrane blebbing, chromatin fragmentation, and increased orange-red fluorescence, confirming apoptosis induction. Treatment with Moringa oleifera extract reduced apoptotic cellular morphology in a dose-dependent manner. Cells pretreated with 100 µg/mL extract demonstrated predominantly viable green fluorescent nuclei with minimal apoptotic alterations. The anti-apoptotic activity of the extract may be associated with inhibition of oxidative stress-mediated mitochondrial injury and suppression of caspase-dependent cell death pathways.

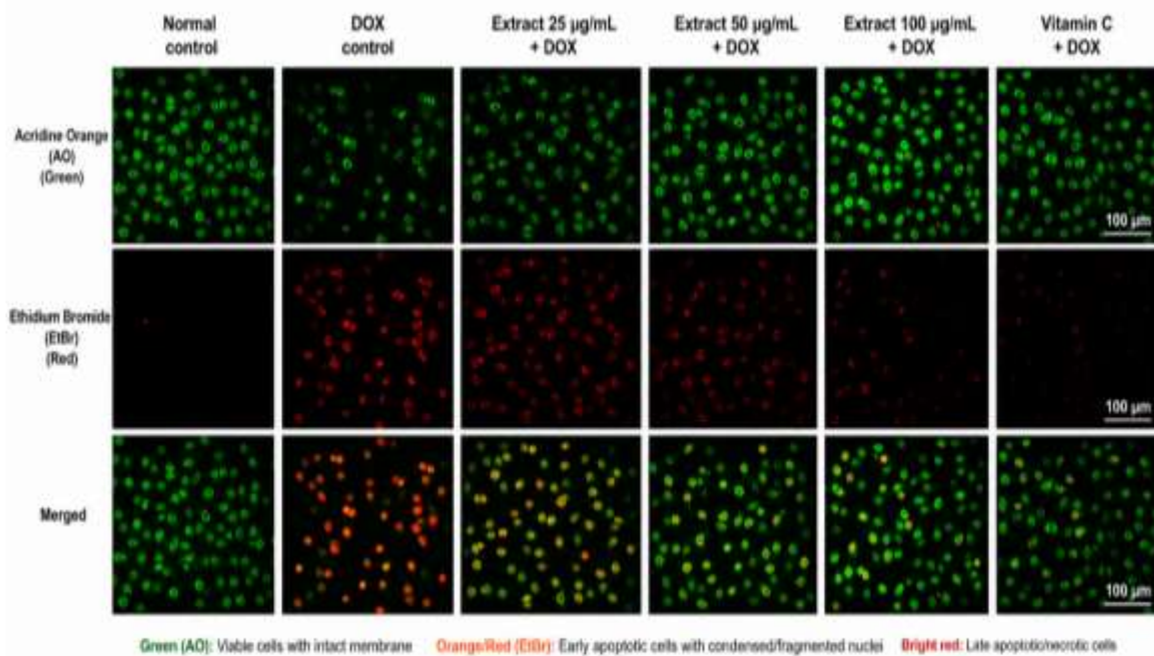

Figure 5. AO/EtBr Fluorescence Images Showing Apoptotic Changes in H9c2 Cells.

3.7 Effect on Caspase-3 Activity

Doxorubicin significantly elevated caspase-3 activity in H9c2 cells, indicating activation of apoptosis execution pathways. The DOX control group demonstrated caspase-3 activity of 3.84 ± 0.16 fold relative to normal cells. Pretreatment with Moringa oleifera extract significantly reduced caspase-3 activation in a concentration-dependent manner. The highest extract concentration reduced caspase-3 activity to 1.48 ± 0.08 fold, which was comparable to vitamin C-treated cells. The reduction in caspase-3 activity indicated effective inhibition of apoptosis-mediated cardiomyocyte death. These findings further supported the mitochondrial protective role of the extract.

Table 5. Effect of Moringa oleifera Extract on Caspase-3 Activity

Group	Caspase-3 Activity (Fold Change)
Normal control	1.00 ± 0.00
DOX control	3.84 ± 0.16
Extract 25 µg/mL + DOX	2.96 ± 0.13
Extract 50 µg/mL + DOX	2.14 ± 0.10
Extract 100 µg/mL + DOX	1.48 ± 0.08
Vitamin C + DOX	1.36 ± 0.07

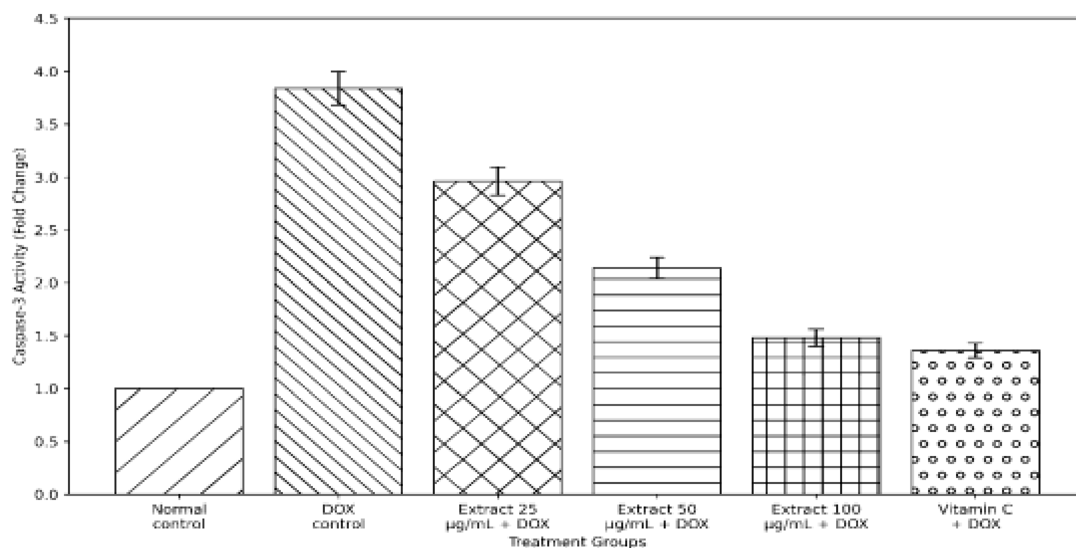

Figure 6. Effect of Moringa oleifera Extract on Caspase-3 Activity in H9c2 Cells.

3.8 Effect on Cellular Antioxidant Biomarkers

Doxorubicin-induced oxidative stress caused significant depletion of endogenous antioxidant defense systems in H9c2 cardiomyoblast cells. The DOX-treated group demonstrated markedly reduced superoxide dismutase,

catalase, and glutathione levels along with substantial elevation of malondialdehyde concentration, indicating severe oxidative membrane damage and lipid peroxidation. Pretreatment with Moringa oleifera extract significantly restored intracellular antioxidant enzyme levels in a concentration-dependent manner. The highest extract concentration demonstrated near-normalization of antioxidant status. Superoxide dismutase activity increased from 8.42 ± 0.38 U/mg protein in the DOX control group to 16.84 ± 0.61 U/mg protein in the extract-treated group. Similarly, catalase and glutathione levels were significantly elevated following extract pretreatment. Malondialdehyde concentration, which reflects lipid peroxidation intensity, was markedly elevated in doxorubicin-treated cells. However, pretreatment with Moringa oleifera extract significantly reduced MDA levels, indicating suppression of oxidative membrane injury. The antioxidant activity observed in the present investigation may be attributed to polyphenolic constituents including flavonoids and phenolic acids present in the extract. These phytoconstituents are capable of scavenging reactive oxygen species, chelating metal ions, stabilizing cellular membranes, and enhancing endogenous antioxidant defence mechanisms. The findings strongly indicated that attenuation of oxidative stress represents one of the primary cardioprotective mechanisms of Moringa oleifera against anthracycline-induced toxicity.

Table 6. Effect of Moringa oleifera Extract on Oxidative Stress Biomarkers in H9c2 Cells

Parameter	Normal Control	DOX Control	Extract 25 µg/mL + DOX	Extract 50 µg/mL + DOX	Extract 100 µg/mL + DOX	Vitamin C + DOX
SOD (U/mg protein)	18.62 ± 0.64	8.42 ± 0.38	11.83 ± 0.42	14.96 ± 0.56	16.84 ± 0.61	17.42 ± 0.58
Catalase (U/mg protein)	42.16 ± 1.42	18.63 ± 0.81	25.74 ± 0.96	33.58 ± 1.12	38.46 ± 1.28	39.82 ± 1.31
GSH (µmol/mg protein)	8.16 ± 0.32	3.21 ± 0.14	4.86 ± 0.18	6.42 ± 0.24	7.38 ± 0.27	7.64 ± 0.25
MDA (nmol/mg protein)	1.82 ± 0.08	5.96 ± 0.22	4.48 ± 0.18	3.14 ± 0.13	2.26 ± 0.10	2.04 ± 0.09

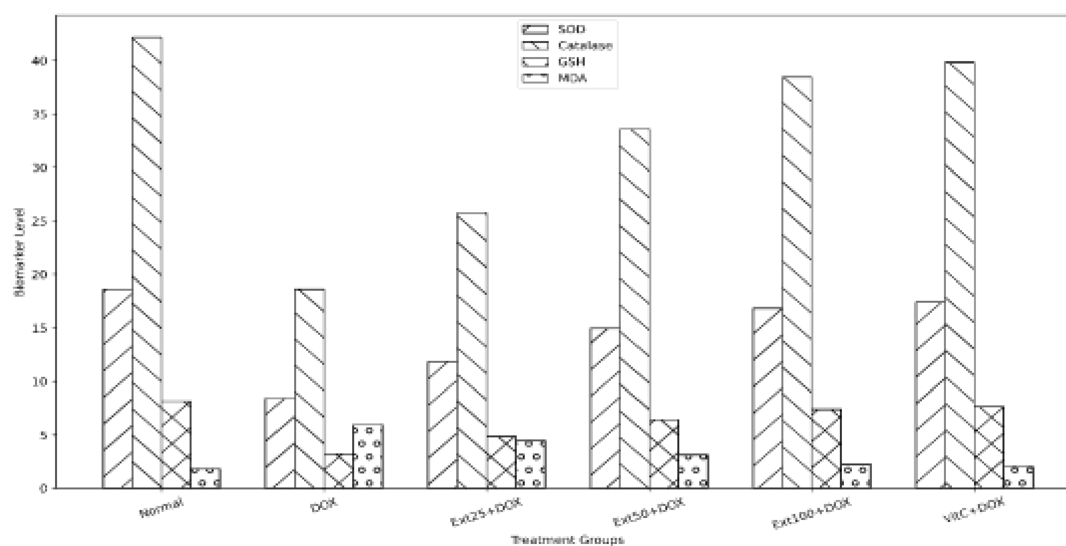

Figure 7. Effect of Moringa oleifera Extract on Cellular Antioxidant Biomarkers in H9c2 Cells.

3.9 Morphological Assessment of H9c2 Cells

Microscopic examination of untreated H9c2 cells revealed normal elongated spindle-shaped morphology with intact cellular architecture and proper intercellular attachment. In contrast, doxorubicin-treated cells exhibited severe morphological alterations including cell shrinkage, membrane blebbing, loss of adherence, cytoplasmic vacuolization, and reduced cell density. Pretreatment with Moringa oleifera extract markedly preserved normal cellular morphology in a concentration-dependent manner. Cells treated with higher extract concentrations demonstrated improved cellular integrity, reduced shrinkage, and restoration of normal spindle-shaped appearance. The observed morphological protection further confirmed the cytoprotective effect of the extract against doxorubicin-induced cardiomyocyte injury.

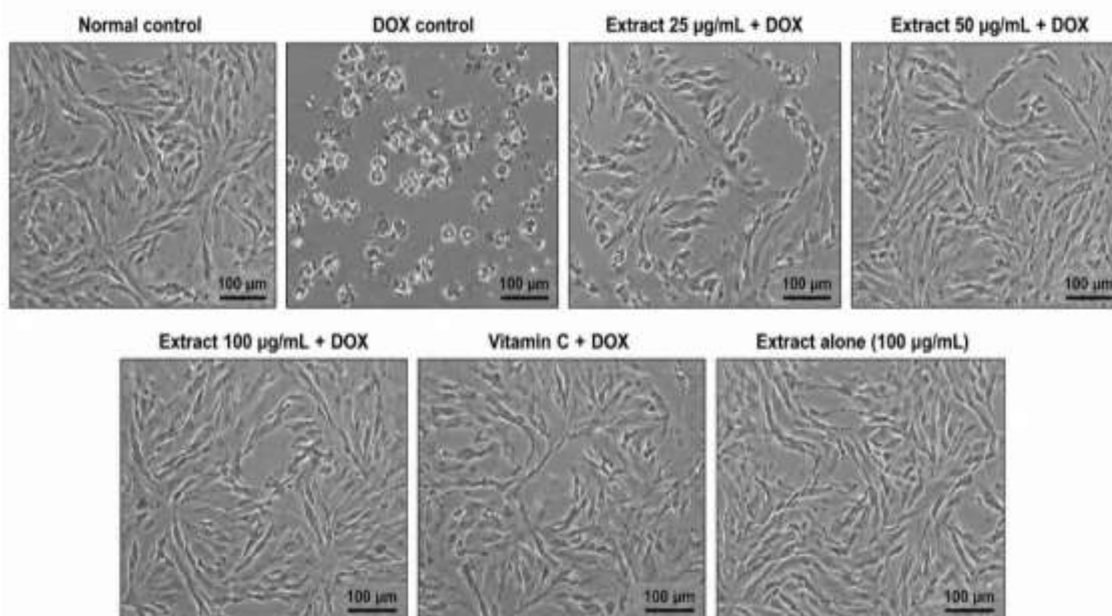

Figure 8. Representative Morphological Images of H9c2 Cells Following Different Treatments.

3.10 Proposed Mechanism of Cardioprotective Action

Based on the obtained experimental findings, the cardioprotective activity of Moringa oleifera leaf extract against doxorubicin-induced toxicity may involve multiple interconnected mechanisms. Doxorubicin exposure induces excessive intracellular reactive oxygen species generation, resulting in mitochondrial membrane depolarization, oxidative damage to lipids and proteins, activation of apoptotic pathways, and eventual cardiomyocyte death. Pretreatment with Moringa oleifera extract significantly suppressed ROS accumulation and restored endogenous antioxidant defence systems including superoxide dismutase, catalase, and glutathione. The extract further preserved mitochondrial membrane potential and inhibited activation of caspase-3-mediated apoptosis. Polyphenolic constituents present in the extract may stabilize mitochondrial membranes, reduce oxidative stress, inhibit cytochrome c release, and attenuate apoptotic signalling pathways. The combined antioxidant and anti-apoptotic actions ultimately contributed to enhanced survival of H9c2 cardiomyoblast cells against anthracycline-induced injury.

4. Conclusion

The present investigation demonstrated significant cardioprotective activity of ethanolic leaf extract of Moringa oleifera against doxorubicin-induced cytotoxicity in H9c2 cardiomyoblast cells. The extract exhibited strong antioxidant and anti-apoptotic effects through suppression of intracellular reactive oxygen species generation, restoration of endogenous antioxidant defence systems, preservation of mitochondrial membrane integrity, and inhibition of caspase-3-mediated apoptosis. Pretreatment with the extract significantly improved cell viability and reduced oxidative stress-associated cellular injury in a concentration-dependent manner. The observed cardioprotective effects may be attributed to the rich phenolic and flavonoid content of the extract, which contributed to stabilization of cellular redox homeostasis and attenuation of mitochondrial dysfunction. The findings suggested that Moringa oleifera possesses considerable therapeutic potential as a natural cardioprotective agent against anthracycline-induced cardiac toxicity. Further investigations involving molecular signalling studies and in vivo experimental models are warranted to validate its clinical applicability in prevention of chemotherapy-associated cardiotoxicity.

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