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Original Article

Cardioprotective Potential of Bitter Melon (*Momordica charantia*) Essential Oil: A Zebra Fish Model Study on Donepezil-HCl Induced Cardiotoxicity with Biochemical Analysis

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Abstract

Background: Drug-induced cardiotoxicity is a major limitation in long-term pharmacotherapy and is often mediated through oxidative stress and inflammatory pathways. Donepezil, a widely prescribed acetylcholinesterase inhibitor, has been reported to induce adverse cardiovascular effects. **Methods:** The present study evaluated the cardioprotective potential of hexane-extracted *Momordica charantia* essential oil (MCEO) against Donepezil-induced cardiotoxicity using a zebrafish (Danio rerio) model. Antioxidant activity was assessed using DPPH radical scavenging and total antioxidant capacity assays, while anti-inflammatory activity was evaluated by red blood cell membrane stabilization. Gene expression of myeloperoxidase (MPO) and heat shock protein 70 (HSP70) was analyzed using RT-PCR in zebrafish heart tissue. **Results:** MCEO demonstrated significant free radical scavenging activity and concentration-dependent enhancement of total antioxidant capacity. The essential oil also exhibited marked anti-inflammatory activity by stabilizing erythrocyte membranes. Donepezil exposure significantly increased MPO expression and reduced HSP70 expression, indicating enhanced oxidative stress and inflammation. Treatment with MCEO significantly reduced MPO expression and restored HSP70 levels toward normal. **Conclusion:** The findings demonstrate that *Momordica charantia* essential oil exerts cardioprotective effects against Donepezil-induced cardiotoxicity through antioxidant and anti-inflammatory mechanisms. MCEO shows promise as a natural cardioprotective agent against drug-induced cardiac injury.

***Keywords:** Cardiotoxicity; Momordica Charantia; Oxidative Stress; Zebrafish Model*

Introduction

Cardiovascular diseases (CVDs) are a major global health threat, contributing to nearly 17.9 million deaths annually, accounting for 32% of all global deaths (World Health Organization, 2021). These diseases include coronary artery disease, heart failure, hypertension, and cardiomyopathies, which significantly impact morbidity and quality of life. Drug-induced cardiotoxicity refers to structural or functional disorders to heart resulting from exposure to specific medications, including chemotherapeutics, antihypertensives, and neuroactive compounds (Mladěnka *et al*., 2018). Among them, Donepezil, a commonly prescribed acetylcholinesterase inhibitor for Alzheimer's disease, has been associated with cardiovascular adverse effects including bradycardia, atrioventricular block, and QT interval prolongation in clinical case reports and safety reviews, suggesting the need for careful cardiac monitoring in susceptible individuals (Tanaka *et al.* 2009).

The adverse effects of synthetic drugs on cardiovascular health necessitate the exploration of natural compounds that offer protective benefits without toxic side effects. In recent years, plant-derived bioactive compounds have gained considerable attention due to their cardioprotective potential. Many

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medicinal plants exhibit antioxidant, anti-inflammatory, and anti-apoptotic properties that help mitigate drug-induced toxicity (Lobo *et al.*, 2010). Among these, *Momordica charantia* (bitter melon) has been traditionally used for various therapeutic purposes, including diabetes management, metabolic disorders, and cardiovascular health (Grover & Yadav, 2004). The essential oil extracted from *M. charantia* (MCEO) is particularly rich in bioactive constituents such as flavonoids, terpenoids, and phenolic compounds, which have demonstrated strong cardioprotective effects by reducing oxidative stress and inflammation (Ahmad *et al.*, 2016).

Zebrafish (*Danio rerio*) have emerged as a widely used model organism for studying cardiovascular development, disease mechanisms, and drug toxicity. Their genetic similarity to humans, transparent embryos, and rapid development make them an ideal choice for cardiovascular research (MacRae & Peterson, 2015). The zebrafish heart shares key physiological and molecular pathways with the human heart, enabling researchers to evaluate drug-induced cardiotoxic effects and potential therapeutic interventions effectively. Studies have demonstrated that zebrafish models can be used to assess cardiac function, oxidative stress responses, and gene expression changes in response to pharmaceutical and environmental toxins (Coppola *et al.*, 2023). Given its advantages, the zebrafish model provides a valuable platform for investigating the cardioprotective effects of *M. charantia* essential oil against Donepezil-HCl-induced cardiotoxicity.

Donepezil-HCl, a cholinesterase inhibitor widely prescribed for Alzheimer's disease, exerts its therapeutic effects by enhancing cholinergic neurotransmission in the brain. However, its impact on cardiac function is a growing concern. Studies have reported that donepezil can lead to adverse cardiovascular effects such as bradycardia and QT interval prolongation, potentially increasing the risk of arrhythmias and sudden cardiac death (Malone & Hancox, 2020).The cardiotoxic effects of Donepezil are attributed to its interaction with the autonomic nervous system, leading to excessive parasympathetic stimulation and impaired cardiac electrophysiology (Huang *et al.*, 2020). Additionally, oxidative stress and mitochondrial dysfunction have been implicated in Donepezil-induced myocardial damage, further highlighting the need for protective strategies (Khuanjing *et al.*, 2021). While previous studies have explored the cardioprotective properties of *Momordica charantia* using crude or polar extracts, no study to date has investigated the hexane-extracted essential oil against Donepezil-HCl–induced cardiotoxicity in a zebrafish model. Furthermore, the integration of biochemical antioxidant assays with molecular markers (MPO and HSP70) provides new mechanistic insight into its cardioprotective action.

Material and Methods

Sample Preparation

To prepare the sample fresh or dried *Momordica charantia* plant material, such as seeds, pulp, or peel have been collected, based on the desired extract. The material has been washed thoroughly with distilled water to remove impurities and air dried in a shaded area to preserve its bioactive properties. Once dried, it has been grinded using a mortar and pestle to break it into smaller pieces, to increase the surface area for more efficient extraction.

Solvent Extraction

Weighed 100 g of the prepared *Momordica charantia* plant material and transferred it into a clean glass container. Hexane was added as the extraction solvent in a 1:5 ratio (500 mL for 100 g of plant material) (Kamalambigeswari *et al.*, 2024). The mixture has been soaked at room temperature for 12–24 hours, stirring occasionally to ensure uniform solvent penetration and efficient extraction.

Filtration

Following extraction, the hexane–plant mixture was filtered using filter paper to separate the plant debris from the hexane–oil extract.

Solvent Removal

The filtered solution was transferred to a rotary evaporator and the temperature was maintained at 40–50 °C to gently evaporate the hexane under reduced pressure (boiling point ~69 °C) (Azwanida, 2015). The remaining residue constituted the extracted essential oil.

Purification and Collection

The extracted oil was collected and stored in an airtight container at 4 °C for further analysis without any additional purification.

Phytochemical Analysis

The tests are qualitative phytochemical tests used to detect the presence of various bioactive compounds in plant extracts. These tests aim to identify specific groups of secondary metabolites, which are responsible for medicinal, antioxidant, antimicrobial, and other biological properties (Islam *et al.*, 2011).

Wagner's Test (Test for Alkaloids):

The Wagner's test is used to detect alkaloids, nitrogen-containing compounds with notable pharmacological properties such as pain relief and antimicrobial effects. A few drops of Wagner's reagent were added to 2–3 mL of the plant extract and observed for the formation of a reddish-brown precipitate, indicating the presence of alkaloids.

Alkaline Reagent Test (Test for Flavonoids):

The test for flavonoids is conducted to detect these polyphenolic compounds known for their antioxidant, anti-inflammatory, and cardioprotective properties. 2 mL of 1 N sodium hydroxide solution was mixed with 2 mL of the plant extract and observed for any color change (Shaikh & Patil, 2020). The formation of a yellow coloration that becomes colorless upon the addition of dilute acid indicated the presence of flavonoids.

Ferric Chloride Test (Test for Phenolic Compounds):

The test for phenolic compounds identifies these potent antioxidants known for their antimicrobial and anti- inflammatory properties. 2–3 drops of 5% ferric chloride solution were added to 2 mL of the plant extract and observed for any color change. The appearance of a dark green, blue, or black coloration indicated the presence of phenolic compounds.

Biochemical Assays

DPPH Test:

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a widely used method to assess the antioxidant potential of various samples, including essential oils. The method involves the use of a stable free radical, DPPH, which exhibits a deep violet color in solution and becomes pale yellow when reduced by an antioxidant. For the assay, essential oil samples are prepared in a suitable solvent such as ethanol due to itsamphipathic nature and solvent compatibility. A DPPH solution is prepared by dissolving the reagent inethanol to a final concentration of 0.1 mM and stored in a dark container to avoid light-induced degradation (Sharma & Bhat, 2009). Serial dilutions of the essential oil (e.g., 10, 25, 50, 75, and 100 µg/mL) are prepared to assess dose-dependent activity. In the procedure, 1 mL of each essential oil dilution is mixed with 2 mL of the DPPH solution in a cuvette or test tube. A blank is prepared by mixing the solvent without the essential oil with the DPPH solution. The reaction mixtures are incubated at room temperature in the dark for 30 minutes to ensure stabilization. Absorbance readings are then taken at 517 nm using a UV spectrophotometer.

Data Analysis:

Calculated DPPH Radical Scavenging Activity:

Using the following formula the percentage of scavenging activity is determined:

Scavenging Activity (%) = (Absorbance of blank - Absorbance of sample) / Absorbance of blank X 100

Plot Results:

a) Plot the scavenging activity (%) against the concentration of the essential oil to observe the dose-response relationship.

b) Determine the IC_{50} value (the concentration of essential oil required to scavenge 50% of DPPH radicals).

TAC Test:

The Total Antioxidant Capacity (TAC) test measures the oxidative stress in a sample, such as essential oils. Different concentrations of the essential oil (10, 25, 50, 75, and 100 µg/mL) were prepared and treated with equal volumes of 600 mM sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. After incubation at room temperature for 30 minutes (Kamalambigeswari*et al*., 2018)., the absorbance was measured at 695 nm using a UV spectrophotometer.

Anti-Inflammatory Test

The haemolytic activity of the essential oil was assessed by incubating various concentrations of the sample (10, 25, 50, 75, and 100 µg/mL) with 1 mL of 10% red blood cell (RBC) suspension. The mixtures were incubated in a water bath at 56°C, then rinsed with running water and allowed to cool at room temperature. Following this, the tubes were centrifuged at 2500 rpm for 5 minutes, and the absorbance of the supernatant was measured at 560 nm using a UV spectrophotometer.

Zebrafish Incubation

In this study, zebrafish embryos were allocated into three experimental groups and incubated for 28 days under standardized laboratory conditions. The Control Group consisted of zebrafish maintained in standard E3 medium without any form of treatment. The Treatment Group was exposed to *Momordica charantia* essential oil (MCEO) at a concentration of 100 µg/mL throughout the incubation period. In the Incubation Group, embryos were pre-incubated with MCEO for 24 hours prior to exposure to Donepezil-HCl at a concentration of 50 µM (OECD, 2013). All groups were closely monitored daily to assess survival rate, observe any morphological alterations, and evaluate cardiac function, ensuring a comprehensive analysis of developmental and physiological responses under each condition.

Post Incubation Procedures

Sample Collection

Following the 28-day incubation period, during which zebrafish were maintained on their respective diets (Control, Induction, and Treatment), the sample collection process was initiated. Zebrafish were euthanized using hypothermic shock to minimize stress and ensure ethical handling. Subsequently, the euthanized specimens were placed on a dissection plate and observed under a stereomicroscope to facilitate precise anatomical dissection and accurate tissue retrieval.

Heart Dissection

To isolate the heart, the ventral side of each zebrafish was carefully incised using fine surgical forceps and scissors to access the thoracic cavity. Under the stereomicroscope, the heart was meticulously dissected to avoid any mechanical damage or contamination (Tan *et al*., 2016). Once isolated, the dissected hearts were immediately rinsed in phosphate-buffered saline (PBS) to eliminate residual blood and other impurities, ensuring clean samples for downstream analysis.

Tissue Homogenization

The collected heart tissues were homogenized in appropriate lysis buffers, such as Tris-HCl and TRIzol, to enable the extraction of enzymes, proteins, and nucleic acids. The homogenates were then centrifuged at 10,000–15,000 rpm for 10–15 minutes, effectively separating the cellular debris from the supernatant. This clear supernatant, rich in biomolecules, was subsequently used for various biochemical assays aimed at evaluating the physiological effects of different treatments.

Storage of Homogenates

For biochemical analyses, the prepared homogenates were centrifuged at 10,000 rpm for 10 minutes, and the resulting supernatant was collected and stored at -80°C until further use. For gene expression studies, the homogenized heart tissues were preserved in Trizol reagent and stored at -80°C to maintain RNA integrity for future extraction and quantitative analysis. This dual approach ensured that both protein and gene-level assessments could be performed accurately.

Molecular Analysis Via RT-PCR

Gene Expression Analysis Using RT-PCR

To evaluate the expression of genes associated with oxidative stress and inflammation, reverse transcription polymerase chain reaction (RT-PCR) was conducted on RNA extracted from zebrafish heart tissues. This molecular approach enabled the quantification of gene transcripts, providing insights into the cellular response to treatment and stress conditions. Total RNA was isolated using the TRIzol method, which is widely used for its efficiency in extracting high-quality RNA suitable for downstream applications such as RT-PCR.

Materials and Reagents

The materials required for RNA extraction included TRIzol reagent, chloroform, isopropanol, 75% ethanol, and DEPC-treated water. Additionally, essential equipment such as centrifuge and sterile microcentrifuge tubes were used to perform the protocol under RNase-free conditions to ensure sample integrity.

RNA Extraction Procedure

The extraction process began with the homogenization of 50–100 mg of zebrafish heart tissue in 1 mL of TRIzol reagent. The homogenized samples were then incubated at room temperature for 5 minutes to facilitate the complete dissociation of nucleoprotein complexes. For phase separation, 0.2 mL of ice-cold chloroform was added per 1 mL of TRIzol, followed by vigorous shaking for 15 seconds and incubation at room temperature for an additional 15 minutes. The samples were then centrifuged at 12,000 g for 15 minutes at 4°C, resulting in the formation of a clear separation between the aqueous phase and organic layers.

RNA Precipitation and Washing

The aqueous phase, containing the RNA, was carefully transferred to a new tube, and RNA was precipitated by adding 0.5 mL of isopropanol per 1 mL of TRIzol. After a 10-minute incubation at room temperature, the samples were centrifuged again at 12,000 g for 8 minutes at 4°C, yielding a visible gel-like white RNA pellet. This pellet was then washed with 1 mL of 75% ethanol and centrifuged at 7,000 g for 5 minutes at 4°C. The ethanol was removed, and the pellet was briefly air-dried for approximately 5 minutes.

RNA Dissolution

The purified RNA pellet was dissolved in 25 µL of DEPC-treated water and incubated at 55–60°C for 10–15 minutes to ensure complete dissolution. In some cases, 30 µL of sterile water was used as an alternative. The extracted RNA was then ready for use in RT-PCR to assess the differential expression of target genes involved in oxidative stress and inflammatory pathways.

cDNA Synthesis

cDNA synthesis was performed using the extracted RNA as a template to generate complementary DNA for gene expression analysis. This process involved the use of reverse transcriptase enzyme, which converts RNA into single-stranded cDNA. The resulting cDNA was then used in RT-PCR to accurately quantify the expression levels of target genes.

Gene Expression Analysis

RT-PCR was carried out using SYBR Green Master Mix to analyze the gene expression levels associated with inflammation and cellular stress response in zebrafish heart tissues. Specifically, the expression of Myeloperoxidase (MPO) and Heat Shock Protein 70 (HSP70) genes was evaluated. MPO serves as a key marker of inflammation and oxidative stress, often contributing to cardiac tissue damage. In contrast, HSP70 functions as a molecular chaperone that plays a protective role in the heart, aiding in the cellular response to stress. To ensure accurate quantification, GAPDH was used as an internal control gene for normalization of the expression data (Fig. 1).

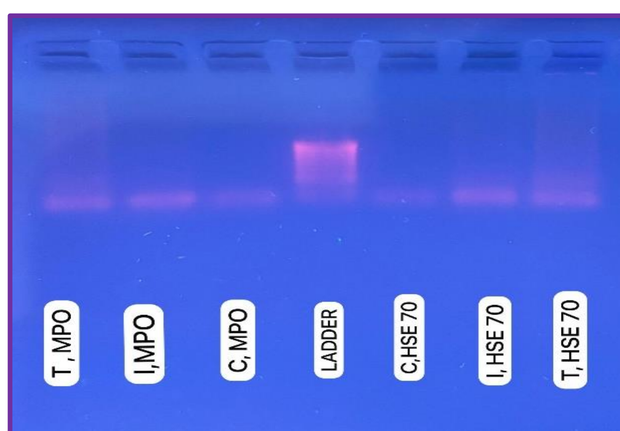

Figure 1: *Gel Running of Expressed Genes*

Results

Phytochemical Analysis

Preliminary qualitative phytochemical analysis of *Momordica charantia* essential oil (MCEO) revealed the presence of key bioactive compounds, as summarized in Table 1.

Wagner's Test (Alkaloids):

The appearance of a reddish-brown precipitate upon the addition of Wagner's reagent to the plant extract confirmed the presence of alkaloids.

Alkaline Reagent Test (Flavonoids):

The formation of a yellow color that disappeared upon the addition of diluted acid indicated the presence of flavonoids in the extract.

Ferric Chloride Test (Phenolic Compounds):

A dark green coloration observed after the addition of ferric chloride confirmed the presence of phenolic compounds.

Table 1: *Phytochemical Screening of MCEO (Qualitative)*

Phytochemical	Test Performed	Result
Alkanoids	Wagner's Test	+
Flavanoids	Alkaline Reagent Test	+
Phenolics	Ferric Chloride Test	+

Biochemical Assays

DPPH Test:

The free radical scavenging activity of MCEO was evaluated using the DPPH assay, and the results are presented in Table 2. MCEO exhibited substantial radical scavenging activity across all tested concentrations, with percentage inhibition ranging from 55.6% to 71.1%. Although the response was not strictly dose-dependent, higher concentrations showed notable scavenging efficiency.

Table 2: *DPPH Radical Scavenging Activity of MCEO*

Concentration (µg/mL)	% Inhibition
10	70.2
25	60.4
50	65.3
75	71.1
100	55.6

Values are expressed as mean of three independent experiments (n = 3).

TAC Test:

The total antioxidant capacity of MCEO increased progressively with concentration, as shown in Table 3. At lower concentrations, MCEO exhibited modest antioxidant activity, which increased markedly at higher concentrations, reaching a maximum activity of 75.3% at 100 µg/mL.

Table 3: *Total Antioxidant Capacity (TAC) of MCEO*

Concentration (µg/mL)	% Activity
10	10.2
25	29.8
50	49.6
75	64.9
100	75.3

Values are expressed as mean of three independent experiments (n = 3).

Anti-Inflammatory Test

The anti-inflammatory potential of MCEO was assessed using the red blood cell (RBC) membrane stabilization assay, and the results are summarized in Table 4. MCEO demonstrated significant membrane stabilization activity across all tested concentrations, with the highest percentage inhibition observed at lower concentrations.

Table 4: *Anti-inflammatory Activity of MCEO by RBC Membrane Stabilization Assay*

Concentration (µg/mL)	% Inhibition
10	90.1
25	75.4
50	68.2
75	60.5
100	55.0

Values are expressed as mean of three independent experiments (n = 3).

Gene Expression Analysis

The expression levels of Myeloperoxidase (MPO) and Heat Shock Protein 70 (HSP70) were analyzed to evaluate Donepezil-HCl–induced cardiotoxicity and the protective effects of MCEO treatment. MPO expression was elevated in the Donepezil-treated group, indicating increased oxidative and inflammatory stress, while HSP70 expression showed stress-related alterations. Treatment with MCEO resulted in reduced MPO expression and increased HSP70 expression, suggesting attenuation of oxidative stress and improved cellular stress response. Quantitative fold-change analysis relative to control is presented in Table 5

Table 5: *Gene Expression Analysis of Inflammatory and Stress Marker (RT-PCR)*

Gene	Control	Donepezil	Donepezil+ MCEO
MPO	1.00	2.45	1.32
HSP70	1.00	0.62	1.85

Values are expressed as mean ± SD of three independent experiments (n = 3).

Discussion

The present study evaluated the cardioprotective potential of *Momordica charantia* essential oil against Donepezil-HCl–induced cardiotoxicity using a zebrafish model. The detection of alkaloids, flavonoids, and phenolic compounds in MCEO supports its biological activity and therapeutic relevance. Flavonoids and phenolic compounds are widely recognized for their antioxidant and anti-inflammatory properties, which contribute to cellular protection against oxidative damage (Yoshime*et al*., 2016).

The strong radical scavenging activity observed in the DPPH assay and the concentration-dependent increase in total antioxidant capacity indicate that MCEO possesses substantial antioxidant potential. These findings suggest that the essential oil can effectively neutralize free radicals and enhance the overall antioxidant defense system. Additionally, the RBC membrane stabilization assay demonstrated notable anti-inflammatory activity, further supporting the protective role of MCEO in preventing membrane damage under inflammatory conditions. These findings are consistent with previous reports highlighting the anti-inflammatory and disease-modulating potential of *Momordica charantia* as a nutraceutical agent (Bortolotti *et al*., 2019)

At the molecular level, Donepezil-HCl exposure resulted in increased MPO expression, reflecting enhanced oxidative stress and inflammation, while alterations in HSP70 expression indicated activation of cellular stress responses. Treatment with MCEO significantly modulated these stress-responsive genes by downregulating MPO and upregulating HSP70, suggesting reduced inflammatory burden and enhanced cardiomyocyte resilience.

The zebrafish model is increasingly recognized as a robust and translational system for evaluating cardiac toxicity and cardioprotective interventions due to its conserved cardiovascular physiology and molecular signaling pathways (Angom & Nakka, 2024). Recent studies have further validated ex vivo and in vivo zebrafish heart models for oxidative stress and cardiotoxicity assessments, highlighting their relevance for mechanistic cardiovascular research (Wu *et al*., 2026). Moreover, alterations in stress-responsive gene expression observed in the present study are consistent with previously reported molecular adaptations during cardiac injury and regeneration in zebrafish (Dhillon-Richardson *et al*., 2025).

While earlier studies have demonstrated the cardiovascular benefits of *M. charantia* and its bioactive constituents, including momordicine-based compounds (Kao *et al*., 2024), most investigations have focused on crude or polar extracts. The present findings extend existing knowledge by demonstrating that the hexane-extracted essential oil fraction of *M. charantia* effectively mitigates Donepezil-HCl–induced cardiotoxicity through combined antioxidant, anti-inflammatory, and molecular stress-regulatory mechanisms.

Limitation

This study is limited to a zebrafish model, which may not fully represent human cardiovascular physiology. Furthermore, the analysis was restricted to selected biochemical and molecular markers, and detailed pathway-level investigations were not performed.

Future Scope

Future studies should focus on elucidating the detailed molecular mechanisms underlying the cardioprotective effects of *Momordica charantia* essential oil, particularly its role in oxidative stress and inflammatory pathways. Additionally, validation in mammalian models and clinical settings is required to establish its therapeutic potential and translational applicability.

Conclusion

This study demonstrates the cardioprotective potential of *Momordica charantia* essential oil (MCEO) against Donepezil-HCl-induced cardiotoxicity in a zebrafish model. Although the cardioprotective properties of *Momordica charantia* have been widely reported, most studies have focused on crude or polar extracts and on classical cardiotoxic agents. The present study is novel in that it evaluates the cardioprotective potential of hexane-extracted essential oil of *M. charantia* against Donepezil-HCl–induced cardiotoxicity using a zebrafish model. Furthermore, this study integrates biochemical, physiological, and molecular analyses by assessing oxidative stress markers along with MPO and HSP70 gene expression, providing mechanistic insight into the protective effects of the essential oil. The results show that Donepezil exposure leads to significant oxidative stress and inflammatory responses, as evidenced by altered antioxidant capacity and increased expression of inflammatory markers. In contrast, treatment with MCEO effectively mitigated these toxic effects by enhancing antioxidant potential and reducing inflammatory stress, thereby improving cardiac resilience. The biochemical assays confirmed that MCEO exhibits strong free radical scavenging activity, total antioxidant capacity, and membrane stabilization ability, which contribute to its protective role against drug-induced toxicity.

Furthermore, gene expression analysis provided molecular evidence supporting MCEO's role in cardio protection. The downregulation of MPO expression in the MCEO-treated group indicates reduced inflammation, while the upregulation of HSP70 suggests enhanced cellular stress resistance and improved cardiac resilience. These findings suggest that MCEO exerts its protective effects through antioxidant and anti-inflammatory mechanisms, counteracting the oxidative burden and inflammatory response induced by Donepezil. This study highlights the potential of essential oil–based phytotherapeutics as safer adjuncts for managing drug-induced cardiotoxicity, particularly for neuroactive drugs such as Donepezil. Despite these promising results, further investigations are required to explore the long-term efficacy and safety of MCEO in preclinical and clinical settings. Future studies should focus on elucidating the precise molecular mechanisms underlying its cardioprotective effects and evaluating its potential in mammalian model.

Conflict of Interest

The authors declare that they have no competing interests.

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