Indian Journal of Research in Homoeopathy

Manuscript 2427

Mitochondria-mediated apoptotic induction by *Chionanthus virginica* Q: A cytotoxic and oxidative stress-driven approach in HepG2 hepatocellular carcinoma cells

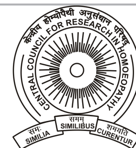
Arun R. Nair

Venkateshan Hariram

Vettrivel Arul

Follow this and additional works at: https://www.ijrh.org/journal

Part of the Homeopathy Commons



Mitochondria-mediated apoptotic induction by *Chionanthus virginica* Q: A cytotoxic and oxidative stress-driven approach in HepG2 hepatocellular carcinoma cells

Abstract

Background: Hepatocellular carcinoma (HCC) is a leading cause of cancer-related deaths worldwide, with current therapeutic options limited by late-stage detection, recurrence, and toxicity. Alternative treatments derived from medicinal plants are gaining attention due to their potential anticancer properties. *Chionanthus virginica*, traditionally used for liver disorders, contains bioactive compounds with hepatoprotective and antioxidant effects. However, its anticancer potential remains largely unexplored. **Objective**: This study evaluates the antioxidant, cytotoxic, and apoptotic effects of *Chionanthus virginica* in mother tincture (Q) and potentised (12C) forms on HepG2 liver cancer cells to determine its efficacy as a natural anticancer agent. **Materials and methods:** HepG2 cells were treated with *Chionanthus virginica* Q and 12C across defined exposure levels (50–1000 µL/mL; extract-equivalent for Q and volume-equivalent for 12C). Antioxidant activity was determined using the DPPH assay, while MTT and LDH assays assessed cytotoxicity. Mitochondrial dysfunction was analysed via MMP and cytochrome c release assays, ROS generation was measured by DCFH-DA fluorescence, and caspase activity was quantified using a colorimetric assay. **Results:** *Chionanthus virginica* Q showed the strongest antioxidant activity (IC_{50} = 101.49 µg/mL) and higher cytotoxicity (CTC_{50} = 330.35 µg/mL), while the response observed with *Chionanthus virginica* 12C was interpreted only as a biological effect under volume-equivalent exposure conditions. One-way and two-way ANOVA were performed for statistical significance. LDH release, cytochrome c translocation, and ROS levels increased significantly ($p < 0.001$), leading to mitochondrial depolarisation and caspase activation, confirming apoptosis. **Conclusion:** This study demonstrates that *Chionanthus virginica* Q induces apoptosis in HepG2 cells via oxidative stress and mitochondrial dysfunction, supporting its potential as a natural anticancer agent. Further studies should focus on bioactive compound isolation and in vivo validation.

INDIAN JOURNAL OF RESEARCH IN HOMOEOPATHY 2026;20(1):45–59

Scan the QR to view the full-text article on the journal website

ORIGINAL ARTICLE

Mitochondria-mediated apoptotic induction by *Chionanthus virginica* Q: A cytotoxic and oxidative stress-driven approach in HepG2 hepatocellular carcinoma cells

Arun R. Nair, Venkateshan Hariram, Vettrivel Arul *

Vinayaka Mission's Research Foundation (Deemed to be University), Salem, Tamil Nadu, India

ABSTRACT

Background: Hepatocellular carcinoma (HCC) is a leading cause of cancer-related deaths worldwide, with current therapeutic options limited by late-stage detection, recurrence, and toxicity. Alternative treatments derived from medicinal plants are gaining attention due to their potential anticancer properties. *Chionanthus virginica*, traditionally used for liver disorders, contains bioactive compounds with hepatoprotective and antioxidant effects. However, its anticancer potential remains largely unexplored. **Objective:** This study evaluates the antioxidant, cytotoxic, and apoptotic effects of *Chionanthus virginica* in mother tincture (Q) and potentised (12C) forms on HepG2 liver cancer cells to determine its efficacy as a natural anticancer agent. **Materials and methods:** HepG2 cells were treated with *Chionanthus virginica* Q and 12C across defined exposure levels (50–1000 μL/mL; extract-equivalent for Q and volume-equivalent for 12C). Antioxidant activity was determined using the DPPH assay, while MTT and LDH assays assessed cytotoxicity. Mitochondrial dysfunction was analysed via MMP and cytochrome c release assays, ROS generation was measured by DCFH-DA fluorescence, and caspase activity was quantified using a colorimetric assay. **Results:** *Chionanthus virginica* Q showed the strongest antioxidant activity ($IC_{50} = 101.49$ μg/mL) and higher cytotoxicity ($CTC_{50} = 330.35$ μg/mL), while the response observed with *Chionanthus virginica* 12C was interpreted only as a biological effect under volume-equivalent exposure conditions. One-way and two-way ANOVA were performed for statistical significance. LDH release, cytochrome c translocation, and ROS levels increased significantly ($p < 0.001$), leading to mitochondrial depolarisation and caspase activation, confirming apoptosis. **Conclusion:** This study demonstrates that *Chionanthus virginica* Q induces apoptosis in HepG2 cells via oxidative stress and mitochondrial dysfunction, supporting its potential as a natural anticancer agent. Further studies should focus on bioactive compound isolation and in vivo validation.

Keywords: Bioactive compounds, *Chionanthus virginica*, Hepatocellular carcinoma, Intrinsic apoptosis, Mitochondrial depolarisation

Introduction

Liver cancer remains a significant global health challenge, with hepatocellular carcinoma (HCC) being the most prevalent and aggressive form.[1] Chronic liver diseases contribute to millions of deaths annually, and HCC is a major contributor to cancer-related mortality worldwide.[2] Various risk factors, including viral hepatitis, excessive alcohol consumption, metabolic disorders, and liver cirrhosis, contribute to the increasing burden of liver cancer.[3] Although early screening, vaccination, and

How to cite this article: Nair AR, Hariram V, Arul V. Mitochondria-mediated apoptotic induction by *Chionanthus virginica* Q: A cytotoxic and oxidative stress-driven approach in HepG2 hepatocellular carcinoma cells. *Indian J Res Homoeopathy.* 2026;20(1):45–59.

Received 26 February 2025; Accepted 16 February 2026.

*Corresponding author.
E-mail address: veldoc4565@gmail.com (V. Arul).

https://doi.org/10.53945/2320-7094.2427

2320-7094/© 2026 Published by Central Council for Research in Homoeopathy (CCRH). This is an open access article under the CC BY-NC-SA 4.0 Licence (https://creativecommons.org/licenses/by-nc-sa/4.0/).

antiviral therapies have reduced the incidence of some hepatic ailments, the search for effective and safer treatments remains a priority.[4] Current therapeutic interventions, including liver transplantation, surgical resection, and systemic chemotherapy, often fail to provide sustained benefits due to late-stage diagnosis, high recurrence rates, and severe side effects.[5] The limitations of conventional treatment strategies have led to an increasing demand for alternative therapeutic approaches that are both effective and minimally toxic.[6] In recent years, natural compounds derived from medicinal plants have garnered attention for their potential anticancer properties.[7] Historically, traditional medicine has played a crucial role in drug discovery, offering bioactive compounds with significant therapeutic value.[8] A substantial number of modern chemotherapeutic agents have originated from natural sources, emphasising the importance of plant-derived compounds in cancer treatment.[9] Among the many medicinal plants, *Chionanthus virginica*, commonly known as fringe tree, has been recognised for its hepatoprotective and therapeutic benefits.[10] Traditionally, it has been used for treating various liver disorders, gallbladder ailments, and inflammatory conditions. The phytochemical constituents of *Chionanthus virginica*, including secoiridoids and lignans, possess notable antioxidant and cytoprotective properties, suggesting its potential in combating oxidative stress-related diseases, including cancer.[11]

Chionanthus virginica has been widely utilised in Homoeopathy, where it is primarily prescribed for liver dysfunction, jaundice, and gallstone-related conditions.[12] The homoeopathic preparations of this plant, particularly in mother tincture (Q) and higher dilutions, have been explored for their therapeutic potential.[13] Homoeopathy follows the principle of potentisation, wherein serial dilution and succussion enhance the medicinal properties of a substance while minimising toxicity.[14] Despite its traditional use and therapeutic claims, the anticancer potential of *Chionanthus virginica* remains largely unexplored in scientific research, particularly in hepatocellular carcinoma models. The growing interest in plant-based therapies for cancer treatment necessitates a thorough evaluation of *Chionanthus virginica* efficacy in targeting liver cancer cells. In vitro models provide a valuable platform for assessing the cytotoxic, apoptotic, and antioxidant properties of medicinal plant extracts.[15] The HepG2 cell line, derived from human liver carcinoma, serves as a well-established model for investigating the effects of potential anticancer agents on liver cancer cells.[16] Evaluating the impact of *Chionanthus virginica* on HepG2 cells can offer insights into its mechanism of action and potential therapeutic applications.

This study aims to investigate the antioxidant, cytotoxic, and apoptotic effects of *Chionanthus virginica* in its Q and potentised (12C) forms on HepG2 cells. Various assays, including free radical scavenging activity, cell viability, lactate dehydrogenase release, mitochondrial membrane potential, cytochrome c release, and caspase activation, are employed to assess its effectiveness. By elucidating the potential of *Chionanthus virginica* as a natural anticancer agent, this study contributes to the ongoing exploration of plant-based therapies for liver cancer, paving the way for future preclinical and clinical investigations.

Materials and methods

The materials used in this study included HepG2 human liver cancer cell lines obtained from National Centre for Cell Sciences (NCCS), Pune, Maharashtra, India, cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 IU/mL *penicillin*, 100 μg/mL *streptomycin*, and 5 μg/mL *amphotericin B* (Gibco, USA) in a 5% CO_2 incubator at 37 °C. For the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, 0.1 mM DPPH solution in methanol and ascorbic acid (standard) were used, with absorbance measured at 514 nm (Shimadzu UV-Visible Spectrophotometer). The 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cytotoxicity assay used MTT reagent (5 mg/mL) and dimethyl sulfoxide DMSO (Sigma Aldrich, USA), with absorbance recorded at 540 nm (Bio-Rad, USA). LDH release was measured using an LDH assay kit (Biogenesis, UK) at 490 nm. Cytochrome c levels were detected via ELISA (Abcam, UK) using isolated mitochondrial and cytosolic fractions. ROS levels were assessed using 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) dye (Thermo Fisher, USA) with fluorescence measured at 485 nm excitation and 530 nm emission (BioTek, USA). Mitochondrial Membrane Potential (MMP) was evaluated using JC-1 dye (Cayman Chemical, USA), with red and green fluorescence measured at 590 nm and 530 nm, respectively. Caspase activity was determined using the Caspase-Glo® 3/7 Assay Kit (Promega, USA) with luminescence detection (Thermo Fisher, USA). *Chionanthus virginica* Q and its 12C potency were procured from Schwabe India Pvt. Ltd., ensuring pharmacopoeial quality standards. This study was designed as a preliminary in-vitro mechanistic investigation focusing on internal comparison between *Chionanthus virginica Q* and its ultra-diluted

12C formulation; therefore, no approved anticancer drug was included as a concurrent positive control.

Quality control and contaminant mitigation

As Chionanthus species may harbour insect-associated residues in the source plant material, additional steps were incorporated to minimise non-plant particulates and potential contaminants prior to bioassays. The mother tincture (Q) was visually inspected, centrifuged at low speed to sediment insoluble debris, and the supernatant was passed through a 0.45 μm pre-filter followed by a 0.22 μm sterile membrane filter before dilution in culture medium. To preferentially reduce highly lipophilic non-plant residues (e.g., cuticular components), an optional defatting clean-up can be performed using hexane partitioning followed by evaporation of residual solvent and reconstitution in ethanol at the original strength prior to working dilutions. For batch-to-batch reproducibility, chromatographic fingerprinting (e.g., HPTLC/GC–MS) is recommended as a quality check to document a consistent phytochemical profile after clean-up.

Assay interpretation and experimental controls

The MTT assay was used as a proxy for metabolic activity/viability and interpreted alongside membrane integrity (LDH release) and apoptosis-linked readouts (cytochrome c release, JC-1 mitochondrial depolarisation, ROS, and caspase-3/7 activation). LDH was interpreted primarily as loss of membrane integrity (late apoptosis/secondary necrosis), and therefore not used as a standalone apoptosis marker. For cytochrome c ELISA, mitochondrial and cytosolic fractions were prepared under cold conditions, and assay steps were performed according to the kit protocol; where feasible, serum-free conditions during the final exposure window are preferred to minimise potential interference. For JC-1 staining and ROS detection, dye loading time, temperature, and protection from light were standardised across groups to reduce protocol-induced variability. Caspase-Glo luminescence was measured with matched blank controls (reagent-only and untreated cells) to account for baseline signal.

DPPH radical scavenging assay

The antioxidant activity of *Chionanthus virginica* Q and *Chionanthus virginica* 12C was evaluated using the DPPH radical scavenging assay, following the method described by Molyneux (2004), with slight modifications. A 100 μM DPPH solution was prepared in methanol, and 1.0 mL of this solution was mixed with 1.0 mL of test samples at varying concentrations (1000, 750, 500, 250, and 50 μg/mL). *Chionanthus virginica* 12C, being an ultra-diluted preparation beyond Avogadro's limit, was evaluated using corresponding volume-equivalent exposures rather than mass-based concentrations. These exposure levels were used only for initial screening; no concentration-based selection or further testing was performed for *Chionanthus virginica* 12C due to its ultra-diluted nature. The reaction mixtures were incubated in the dark at room temperature for 30 minutes to prevent light-induced degradation. After incubation, the absorbance was recorded at 514 nm using a UV-Visible spectrophotometer. To ensure clarity in data interpretation, two control groups were included in the assay. The negative control (DPPH control) consisted of 1.0 mL of DPPH solution mixed with 1.0 mL of methanol, which served as the reference for calculating the percentage inhibition. A vehicle control was also included, consisting of 1.0 mL of DPPH solution mixed with 1.0 mL of ethanol, to account for any potential interference from the solvent used for the test samples.[17] The percentage inhibition of DPPH radicals was calculated using the following formula:

$$\%Inhibiton = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

The absorbance of the DPPH control refers to the absorbance of the negative control (DPPH + methanol), and the absorbance of the sample refers to the test sample or vehicle control. The IC50 value, representing the concentration of the extract required to inhibit 50% of DPPH radicals, was determined using GraphPad Prism 5.0 software.

MTT cytotoxicity assay

The cytotoxic effects of *Chionanthus virginica* Q and *Chionanthus virginica* 12C on HepG2 cells were assessed using the MTT assay, a colorimetric method based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into insoluble formazan by mitochondrial dehydrogenase enzymes in metabolically active cells. This reduction, which occurs only in viable cells, results in a colour change that can be quantified spectrophotometrically. HepG2 cells were procured from NCCS, Pune, Maharashtra, India, and maintained in DMEM supplemented with 10% FBS, 100 IU/mL *penicillin*, 100 μg/mL *streptomycin*, and 5 μg/mL *amphotericin B*. Cells were cultured in a 5% CO_2 incubator at 37 °C and were seeded into 96-well microtiter plates at a density of 1×10^5 cells/mL, followed by overnight

incubation to allow for adherence. Test samples were prepared by dissolving the required quantity of *Chionanthus virginica* Q in DMSO, which was then diluted with DMEM supplemented with 2% FBS to obtain extract-equivalent concentrations. *Chionanthus virginica* 12C, being an ultra-diluted preparation beyond Avogadro's limit, was not expressed in mass-based concentration units and was instead administered as defined volume-equivalent exposures (50, 250, 500, 750, and 1000 μL/mL). The final concentration of DMSO in all experimental wells did not exceed 0.1% (v/v), and vehicle control wells containing equivalent DMSO concentration were included. Ethanol content was standardised across all treatment groups and corresponding vehicle control wells. An untreated control (cells with only culture medium) was also included for comparison. After 24 hours of treatment, 20 μL of MTT reagent (5 mg/mL in phosphate-buffered saline (PBS)) was added to each well, and the plates were incubated for 4 hours at 37°C in the dark to allow the formation of formazan crystals. The medium was then carefully removed, and 100 μL of DMSO was added to each well to dissolve the formazan crystals. The absorbance was recorded at 540 nm using a microplate reader (Bio-Rad, USA).[15] To quantify cytotoxicity, the percentage of cell growth inhibition was calculated using the following formula:

$$\%Growth\ Inhibiton = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

For *Chionanthus virginica* Q, the CTC_{50} value was calculated based on extract-equivalent concentration. For *Chionanthus virginica* 12C, cytotoxic effects were interpreted based on volume-equivalent exposure rather than mass-based CTC_{50} values.

Lactate dehydrogenase (LDH) release assay

Following the MTT assay, the LDH release assay was conducted to further evaluate the cytotoxic effects of *Chionanthus virginica* Q on HepG2 cells by assessing membrane integrity. As *Chionanthus virginica* Q demonstrated higher cytotoxic activity in the MTT assay than *Chionanthus virginica* 12C, subsequent membrane integrity analysis was restricted to *Chionanthus virginica* Q to avoid solvent- or dilution-related confounding effects. An untreated cell line was included as an independent experimental group to observe baseline LDH release and to serve as a reference for membrane integrity under physiological conditions. After 24 hours of treatment, 100 μL of cell culture supernatant was collected from each well to measure LDH leakage. Ethanol content was maintained constant across all treatment and vehicle control groups to exclude solvent-induced membrane damage. The LDH release assay kit (Biogenesis, UK) was used according to the manufacturer's protocol, and absorbance was measured at 490 nm using a microplate reader (Bio-Rad, USA).[18] The percentage of LDH release was calculated using the formula:

$$LDH\ Release\ \% = \frac{Experimental\ LDH\ Release}{Total\ LDH\ Release} \times 100$$

where Experimental LDH Release corresponds to the LDH detected in the supernatant of each treatment group, and Total LDH Release was determined by lysing all cells in a separate well to obtain maximum LDH release.

Cytochrome c oxidase assays

The cytochrome c oxidase assay was conducted to assess mitochondrial respiratory activity in HepG2 cells treated with *Chionanthus virginica* Q at concentrations of 50, 250, 500, 750, and 1000 μL/mL (extract-equivalent exposure), with untreated cells included as an independent experimental group. *Chionanthus virginica* 12C was excluded from this assay because mitochondrial enzyme kinetics are known to require molecular-level substrate interactions; therefore, based on accepted biochemical principles and MTT screening results, *Chionanthus virginica* Q was selected for mechanistic mitochondrial evaluation. After 24 hours of treatment, mitochondria were isolated by differential centrifugation and diluted 1:10 in mitochondrial assay buffer containing 115 mM KCl, 10 mM KH_2PO_4, 2 mM $MgCl_2$, 3 mM HEPES, 1 mM EGTA, and 0.2% BSA, with pH adjusted to 6.8. Ferrocytochrome c (5–30 μM; substrate concentration) was added, and absorbance changes at 550 nm were measured over 10–60 seconds using a Shimadzu UV1650PC spectrophotometer in a 0.25 mM oxygen environment at 25°C. Inhibition studies were conducted using azide (0.1 mM and 1.0 mM) and cyanide (7 μM and 14 μM) as reference inhibitors, varying ferrocytochrome c concentrations to evaluate mitochondrial function. Additionally, cytochrome c release was quantified via ELISA, where mitochondrial and cytosolic protein fractions were loaded into an ELISA plate, incubated with primary and HRP-conjugated secondary antibodies, and absorbance was recorded using a microplate reader (Bio-Rad, USA).[19] The percentage of cytochrome c oxidase activity was calculated based on ferrocytochrome c oxidation, with ELISA confirming cytochrome c release.

Reactive oxygen species (ROS) assay

ROS assay was conducted to evaluate oxidative stress levels in HepG2 cells treated with *Chionanthus virginica* Q at concentrations of 50, 250, 500, 750, and 1000 μL/mL (extract-equivalent exposure), with an untreated cell line included as an independent experimental group. HepG2 cells were seeded into 96-well plates at a density of 1×10^5 cells/well and incubated overnight at 37°C in a 5% CO_2 atmosphere to allow for proper adherence. After incubation, the culture medium was removed, and 100 μL of *Chionanthus virginica* Q at the designated concentrations was added to the respective wells, while untreated control wells contained only culture medium. Ethanol content was maintained constant across all treatment and corresponding vehicle control wells to exclude solvent-induced oxidative effects. Following 24 hours of treatment, the media was removed, and the wells were washed with PBS to remove residual compounds. The ROS-sensitive fluorescent dye 2′,7′-Dichlorofluorescin diacetate (DCFH-DA) (10 μM; probe concentration) was freshly prepared in PBS and added to each well, ensuring light protection to prevent photobleaching. The cells were incubated for 30–60 minutes at 37°C, after which the dye was removed, and wells were washed with PBS before adding fresh PBS for measurement. Fluorescence intensity was recorded at 485 nm excitation and 530 nm emission using a microplate reader (BioTek, USA).[20] The relative ROS levels (%) were calculated using the formula:

$$Relative\ ROS\ Levels\ \% = \frac{Fluorescence\ Intensity\ of\ Treated\ Cells}{Fluorescence\ Intensity\ of\ Control} \times 100$$

where treated cells represent those exposed to *Chionanthus virginica* Q at different concentrations, and control cells refer to the untreated group.

Mitochondrial membrane potential (MMP)

The mitochondrial membrane potential ($\Delta\psi$m) assay was conducted to assess mitochondrial function in HepG2 cells treated with *Chionanthus virginica* Q at concentrations of 50, 250, 500, 750, and 1000 μL/mL (extract-equivalent exposure), with an untreated cell line included as an independent experimental group. After 24 hours of treatment, the cells were washed with PBS to remove residual compounds and incubated with JC-1 dye (10 μM; probe concentration) at 37 °C for 15–30 minutes in the dark to prevent photobleaching. In healthy mitochondria, JC-1 aggregates emit red fluorescence, while in depolarised mitochondria, it remains in a monomeric form emitting green fluorescence. Following incubation, the cells were washed three times with PBS and immediately analysed using a fluorescence microscope and flow cytometer to measure the red-to-green fluorescence intensity ratio, which indicates mitochondrial membrane potential.[21] Ethanol content was maintained constant across all treatment and vehicle control groups to exclude solvent-induced mitochondrial depolarisation. The $\Delta\psi$m was calculated using the formula:

$$\Delta\psi m = \frac{Red\ FL\ sample / Red\ FL\ control}{Green\ FL\ sample / Green\ FL\ control}$$

where Red FL sample and Green FL sample represent the fluorescence intensities in treated cells, while Red FL control and Green FL control refer to intensities in untreated cells.

Caspase activity

The caspase activity assay was performed to evaluate apoptosis induction in HepG2 cells treated with *Chionanthus virginica* Q at concentrations of 50, 250, 500, 750, and 1000 μL/mL (extract-equivalent exposure), with an untreated cell line included as an independent experimental group. After 24 hours of treatment, the cells were washed with cold PBS and lysed using a standard lysis buffer to release intracellular contents. Equal volumes of the cell lysate were transferred to a 96-well plate, and a caspase-specific colorimetric substrate (kit-provided) was added to each well. The reaction mixtures were incubated at 37°C to allow enzymatic cleavage of the substrate. The absorbance was measured at the appropriate wavelength using a microplate reader (Bio-Rad, USA) to determine caspase activity.[22] Ethanol content was maintained constant across all treatment and vehicle control groups to exclude solvent-induced effects on apoptotic signalling. The caspase activity was calculated using the formula:

$$Caspase\ Activity = \frac{Absorbance\ of\ sample - Absorbance\ of\ blank}{Protein\ Concentration\ X\ Time}$$

where absorbance of sample represents the optical density of treated cells, absorbance of blank corresponds to the background absorbance from control wells without caspase activity, protein concentration was determined using the BCA protein assay, and time refers to the incubation period.

Statistical analysis

All experiments were conducted in triplicates, and data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS

software (Version 26.0, IBM Corp., USA) to evaluate the significance of differences between experimental groups. One-way ANOVA was used to assess variations in antioxidant activity (DPPH assay), cytotoxicity (MTT assay), lactate dehydrogenase (LDH) release, ROS generation, MMP, caspase activity. Tukey's post-hoc test was applied for multiple comparisons following ANOVA, while two-way ANOVA was utilised in cytochrome c and ROS assays to examine interactions between treatment type and exposure level. For assays involving *Chionanthus virginica* 12C, statistical comparisons were based on volume-equivalent exposure rather than mass-based concentration. Levene's test was performed to check homogeneity of variances, and necessary corrections were made when $p < 0.05$. Statistical significance was set at $p < 0.05$, with highly significant differences indicated at $p < 0.01$ and $p < 0.001$. Data visualisation and statistical calculations were carried out using GraphPad Prism 5.0 and SPSS software.

Results

The antioxidant potential and cytotoxicity assessment via the DPPH and MTT assays provided the basis for selecting *Chionanthus virginica* Q for further studies. Among the tested samples, *Chionanthus virginica* Q demonstrated the strongest antioxidant activity, with an IC50 value of 101.49 μg/mL, significantly lower than *Chionanthus virginica* 12C (expressed as volume-equivalent exposure) and ethanol (IC50 = 373.24 μg/mL) [Fig. 1]. The superior free radical scavenging ability of *Chionanthus virginica* Q suggests its potential role in modulating oxidative stress, a key factor influencing cancer progression and apoptosis induction. Furthermore, in the MTT assay, *Chionanthus virginica* Q exhibited the highest cytotoxicity against HepG2 cells, with a CTC50 value of 330.35 μg/mL, compared to the biological response observed with *Chionanthus virginica* 12C under volume-equivalent exposure conditions. The significant reduction in cell viability upon treatment with *Chionanthus virginica* Q ($p < 0.001$) indicates its potent anti-cancer potential [Fig. 2].

Given that the primary focus of the study was to identify a sample with both strong antioxidant activity and potent cytotoxicity against HepG2 cells, *Chionanthus virginica* Q was selected for further analyses, including LDH release, mitochondrial dysfunction, ROS production, apoptosis induction, and gene expression studies. *Chionanthus virginica* 12C was not included in the downstream mechanistic assays described below. As an ultra-diluted preparation beyond Avogadro's limit, 12C was evaluated only during the initial DPPH and MTT screening under volume-equivalent exposure. Subsequent assays assessing LDH release, reactive oxygen species generation, mitochondrial membrane potential, cytochrome c release, and caspase activation require molecular-level interactions and were therefore conducted exclusively with *Chionanthus virginica* Q. Accordingly, Figs. 3 to 7 present mechanistic assay data exclusively for *Chionanthus virginica* Q. The LDH assay demonstrated a concentration-dependent increase in LDH release upon treatment with

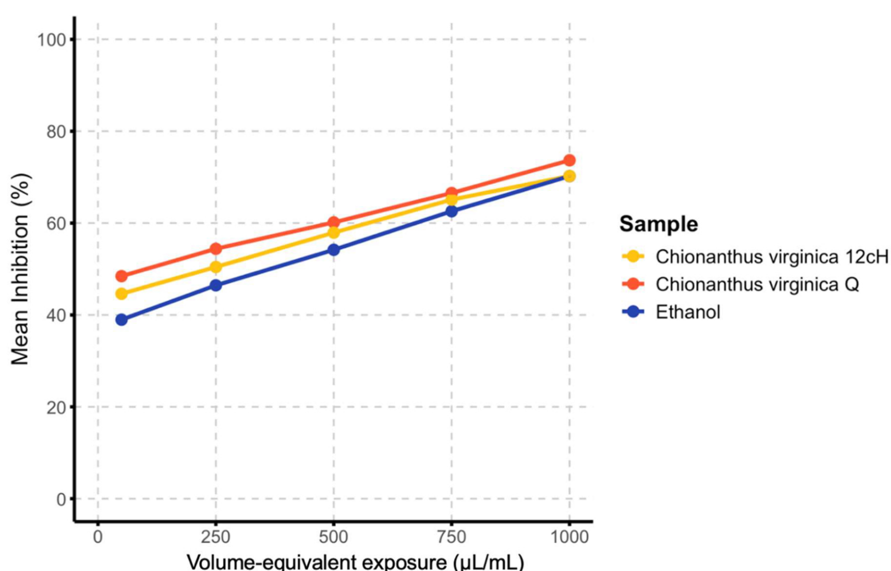


Fig. 1. DPPH assay showing mean inhibition (%) of *Chionanthus virginica* Q, *Chionanthus virginica* 12C, and ethanol at 50–1000 μL/mL, with *Chionanthus virginica* Q showing the highest activity.

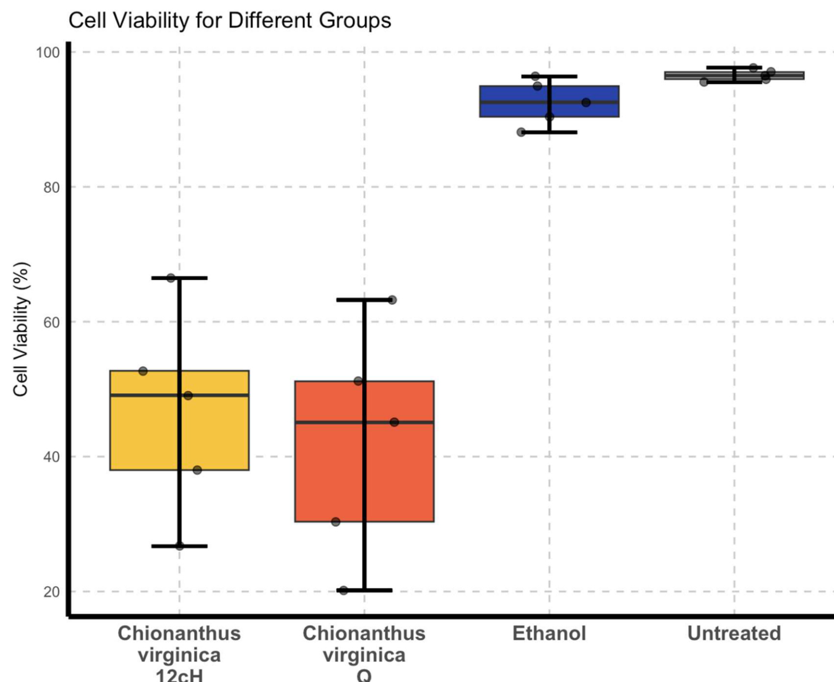


Fig. 2. MTT assay showing cell viability (%) in HepG2 cells treated with *Chionanthus virginica Q*, *Chionanthus virginica* 12C, and ethanol, with the lowest viability observed in *Chionanthus virginica* Q-treated cells.

Chionanthus virginica Q, confirming its role in inducing membrane damage and cytotoxicity. The highest LDH release was observed at 1000 μL/mL (extract-equivalent exposure), with an average release of 83.70 $\pm$ 0.02 AU, significantly higher than the untreated cell line (0.94 $\pm$ 0.03 AU, $p < 0.001$). ANOVA confirmed that both treatment and concentration significantly influenced LDH release ($p < 0.001$) [Fig. 3].

These findings reinforce the conclusion that *Chionanthus virginica* Q disrupts cellular integrity, further supporting its cytotoxic potential. The cytochrome c assay revealed a progressive increase in cytosolic cytochrome c levels following treatment with *Chionanthus virginica* Q, while mitochondrial cytochrome c levels decreased correspondingly. At 1000 μL/mL, cytosolic cytochrome c levels peaked at 86.26 $\pm$ 0.19 AU, whereas mitochondrial cytochrome c declined to 13.74 $\pm$ 0.19 AU. Statistical analysis confirmed that these changes were highly significant ($p < 0.001$) [Fig. 4].

The substantial release of cytochrome c from mitochondria into the cytosol suggests that *Chionanthus virginica* Q induces apoptosis via the mitochondrial pathway. Oxidative stress analysis through the ROS assay further confirmed the cytotoxic effects of *Chionanthus virginica* Q. A significant increase in ROS production was observed in a dose-dependent manner, with relative ROS levels reaching 79.56% at 1000 μL/mL compared to only 2.76% in the untreated control ($p < 0.001$) [Fig. 5]. This suggests that oxidative stress contributes to the cytotoxic mechanism of *Chionanthus virginica* Q.

The MMP assay further confirmed mitochondrial dysfunction in response to *Chionanthus virginica* Q treatment. A progressive decline in the MMP ratio was observed with increasing concentrations, dropping from 1.34 at 50 μL/mL to 1.17 at 1000 μL/mL, while untreated cells exhibited significantly higher MMP values (2.09) [Fig. 6]. ANOVA confirmed a highly significant reduction in MMP following treatment with *Chionanthus virginica* Q ($p < 0.001$), supporting its role in mitochondrial depolarisation and apoptosis induction. Caspase activity measurements provided additional evidence for apoptosis induction. A dose-dependent increase in caspase activity was noted, reaching 0.73 AU at 1000 μL/mL, compared to 0.09 AU in the untreated group ($p < 0.001$) [Fig. 7]. These results confirm that *Chionanthus virginica* Q triggers apoptosis through caspase activation, reinforcing its role as a potential anti-cancer agent.

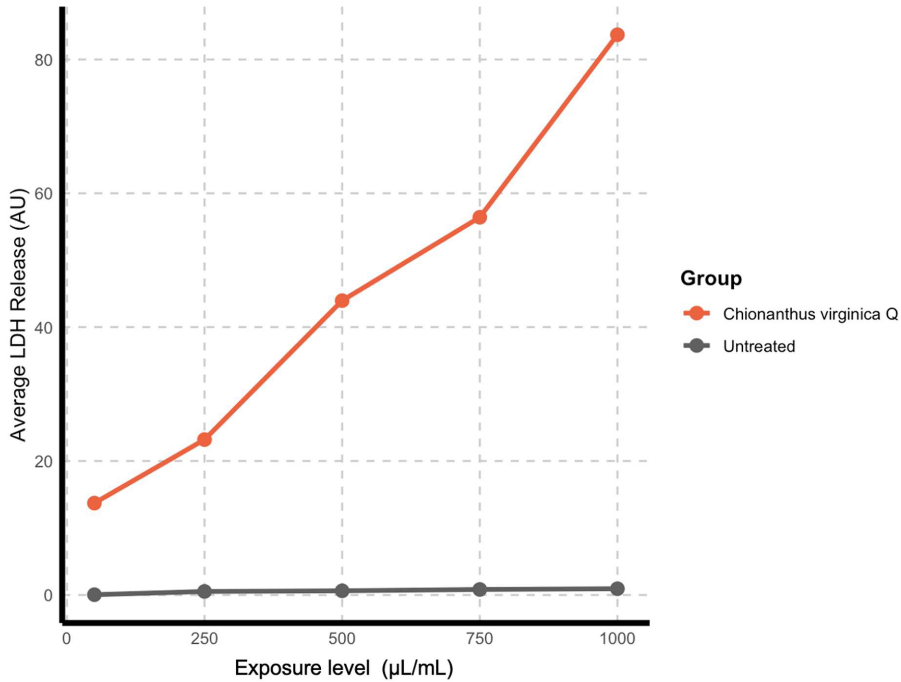


Fig. 3. LDH assay showing average LDH release (AU) in HepG2 cells treated with *Chionanthus virginica* Q and untreated controls, with a dose-dependent increase in LDH release observed in the treated group, indicating higher cytotoxicity.

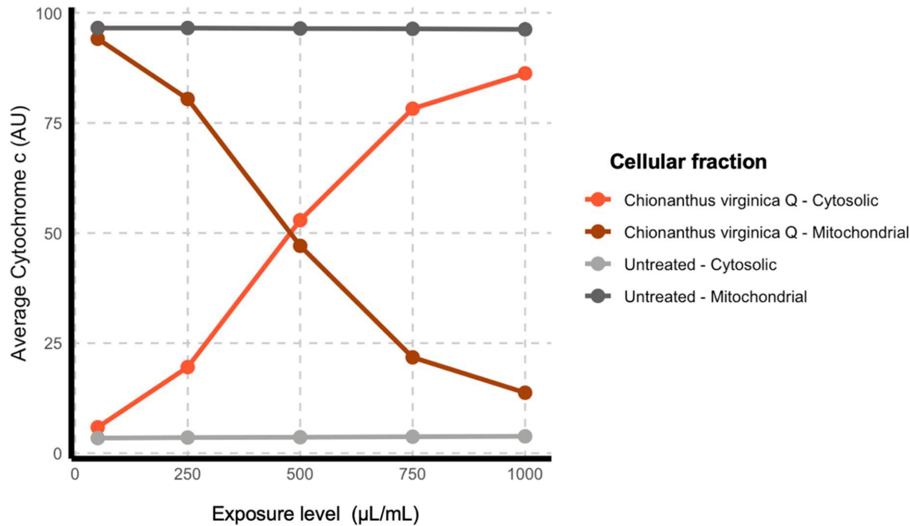


Fig. 4. Cytochrome c oxidase assay showing a concentration-dependent increase in cytosolic cytochrome c and a decrease in mitochondrial cytochrome c in *Chionanthus virginica* Q-treated HepG2 cells, indicating mitochondrial membrane permeabilisation and apoptosis induction.

Discussion

The present study investigates the antioxidant and cytotoxic properties of *Chionanthus virginica* Q on HepG2 cells, revealing significant findings that align with recent research on related species and compounds. In the DPPH radical scavenging assay, *Chionanthus virginica* Q demonstrated a notable IC50 value of 101.49 μg/mL, indicating strong antioxidant activity. This result is consistent with the findings of Mihai *et al.*, who reported that *Chionanthus pubescens* exhibited significant antioxidant activity, with an IC50 value of 62.89 mg/mL in the DPPH assay. Although the species and IC50 values differ, both studies highlight the potent antioxidant capabilities within the *Chionanthus* genus.[10]

The intrinsic mitochondrial apoptosis pathway provides a coherent framework for the pattern

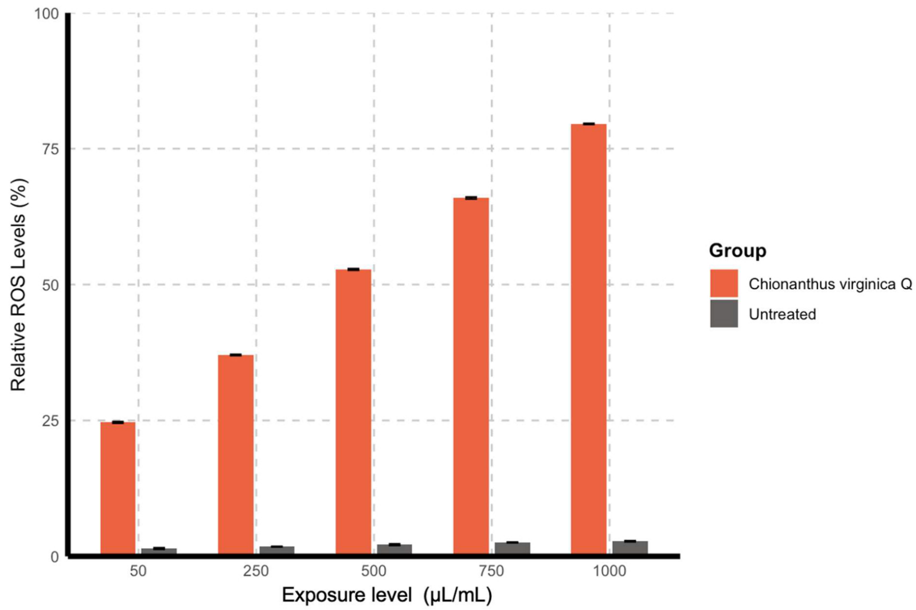


Fig. 5. ROS assay showing a concentration-dependent increase in ROS levels (%) in *Chionanthus virginica* Q-treated HepG2 cells, indicating oxidative stress, while untreated cells exhibit minimal ROS generation.

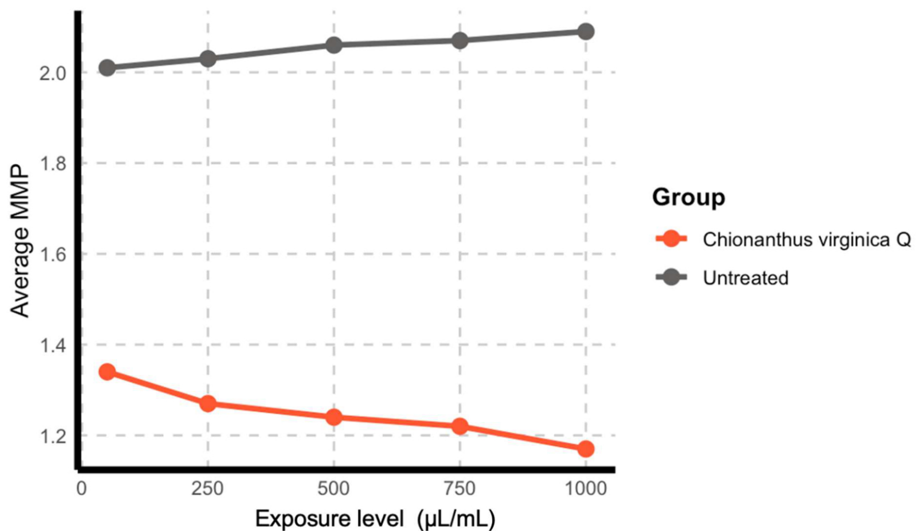


Fig. 6. Mitochondrial membrane potential (MMP) assay showing a concentration-dependent decrease in MMP levels in *Chionanthus virginica* Q-treated HepG2 cells, indicating mitochondrial depolarisation, while untreated cells maintain stable MMP.

observed in this study. Pro-apoptotic stress shifts the balance of Bcl-2 family regulators toward Bax/Bak activation, promoting mitochondrial outer membrane permeabilisation (MOMP) and loss of mitochondrial membrane potential. This event enables cytochrome c release into the cytosol, where it associates with Apaf-1 and ATP/dATP to form the apoptosome complex, leading to initiator caspase-9 activation and subsequent activation of executioner caspases such as caspase-3/7. In our data, elevated intracellular ROS, JC-1–based mitochondrial depolarisation, increased cytochrome c release, and increased caspase-3/7 activity collectively support mitochondria-mediated apoptotic induction by *Chionanthus virginica* Q in HepG2 cells, consistent with a chemotherapy-relevant mechanism that many anticancer agents ultimately converge upon.

The observation that measurable bioactivity occurred predominantly with the mother tincture (Q) is biologically plausible in an in vitro framework because Q contains quantifiable phytochemical constituents capable of engaging cellular redox and apoptotic pathways at the concentrations tested. In

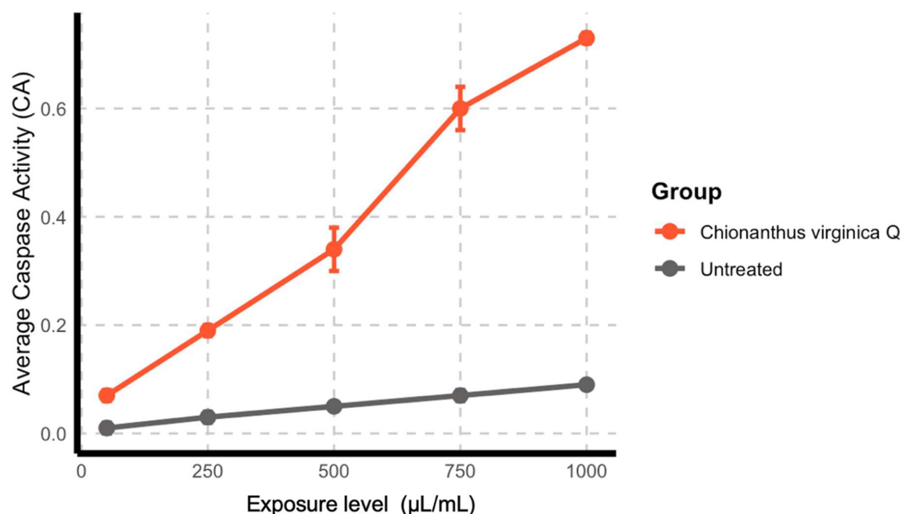


Fig. 7. Caspase activity assay showing a concentration-dependent increase in caspase activity (CA) in *Chionanthus virginica* Q-treated HepG2 cells, indicating apoptotic induction, while untreated cells maintain minimal caspase activation.

contrast, ultra-diluted preparations such as 12C may not retain detectable amounts of the original phytochemicals and therefore may not elicit molecular pharmacological effects in cell-based biochemical assays designed to measure concentration-dependent cytotoxicity and apoptosis. Accordingly, the rationality supported by the present study pertains specifically to the mother tincture as a phytochemical-based candidate for further anticancer evaluation, while emphasising that disease-use claims require additional evidence including selectivity testing in normal cells and in vivo validation.

The cytotoxic effects of *Chionanthus virginica* Q on HepG2 cells, evidenced by a CTC50 value of 330.35 μg/mL, underscore its potential as an anti-cancer agent. In contrast, biological responses observed with *Chionanthus virginica* 12C were interpreted based on volume-equivalent exposure rather than mass-based cytotoxic parameters, consistent with its ultra-diluted nature. Accordingly, the present work was structured as a preliminary mechanistic in-vitro study emphasising internal comparison between test formulations, rather than benchmarking against an approved chemotherapeutic agent. While specific studies on *Chionanthus virginica* are limited, research on related species provides supportive evidence. For instance, a study by Kwak *et al.* on *Chionanthus retusus* identified cytotoxic phenolic compounds that exhibited significant activity against various cancer cell lines, suggesting that bioactive compounds in *Chionanthus* species may contribute to their anti-cancer properties.[23]

Our investigation into mitochondrial dysfunction revealed that *Chionanthus virginica* Q induces mitochondrial depolarisation, as evidenced by a decrease in MMP. This finding aligns with broader research linking mitochondrial dysfunction to apoptosis. A review by Li *et al.* discusses how mitochondrial dysfunction can trigger chronic inflammation and immune disorders, emphasising the role of mitochondria in cell death pathways. Although their focus is on rheumatoid arthritis, the underlying mechanisms are relevant to cancer cell apoptosis induced by mitochondrial impairment.[24]

The observed increase in ROS production following *Chionanthus virginica* Q treatment is indicative of oxidative stress, a known mediator of apoptosis. This is supported by a study on the antioxidant activity of lignans from *Chionanthus virginicus*, which highlights the role of phenolic compounds in modulating oxidative stress.[25] The balance between ROS generation and antioxidant defense is critical, and the exposure-dependent pro-oxidant shift induced by *Chionanthus virginica* Q may contribute to its cytotoxic effects in cancer cells.[26] The release of cytochrome c and the subsequent activation of caspases observed in our study confirm the involvement of the intrinsic apoptotic pathway in *Chionanthus virginica* Q - induced cell death. This mechanism is well-documented in the context of mitochondrial dysfunction leading to apoptosis. A comprehensive review by Yao Zong *et al.* discusses how mitochondrial dysfunction and the release of pro-apoptotic factors like cytochrome c play a central role in the intrinsic apoptotic pathway, contributing to disease progression and potential therapeutic interventions.[27] The findings of this study are in concordance with these established mechanisms, suggesting that *Chionanthus virginica* Q exerts its cytotoxic effects through mitochondrial-mediated apoptosis.

While the present data support cytotoxic and pro-apoptotic effects in HepG2 cells, safety cannot be concluded from an in vitro model. *Chionanthus virginica* Q is a complex ethanolic botanical preparation, and this study did not assess toxicity in normal hepatocytes, systemic tolerability, potential drug–herb interactions, or reproductive and developmental safety. Therefore, adverse-effect risk remains undefined, and use during pregnancy or breastfeeding cannot be recommended on the basis of the current evidence. Future studies should include selectivity testing in normal liver cells, in vivo acute and sub-chronic toxicity, and dedicated reproductive/developmental toxicology, along with benchmarking against standard chemotherapeutic positive controls.

This work has several limitations that should be considered when interpreting the findings. First, the experiments were conducted only in vitro using a single hepatocellular carcinoma cell line (HepG2); therefore, the results cannot be directly extrapolated to in vivo tumour biology or clinical efficacy. Second, no normal hepatocyte/primary liver cell line was included to assess cancer-selective toxicity, and the study did not include a concurrent reference chemotherapeutic positive control, which limits benchmarking of effect size. Third, mechanistic assessments were performed at a single exposure duration (24 h) without pathway-specific inhibitors or genetic validation; therefore, causality within the proposed ROS–mitochondria–caspase axis remains inferential. Additionally, each assay has intrinsic technical constraints (e.g., MTT reflects metabolism, LDH reflects membrane integrity, dye-based ROS/MMP readouts are protocol-sensitive, and luminescent caspase assays can show background signal), so interpretation was based on convergence across multiple endpoints rather than any single assay. Finally, although filtration and debris-removal steps were applied, trace non-plant residues (including potential insect-associated metabolites) cannot be fully excluded without targeted chemical profiling; future work should address this through standardised clean-up and analytical verification.

Conclusion

The present study demonstrates the significant antioxidant and cytotoxic potential of *Chionanthus virginica* Q against HepG2 cells. The DPPH assay confirmed its strong free radical scavenging activity, while the MTT assay revealed potent cytotoxic effects. *Chionanthus virginica* Q induced oxidative stress, as evidenced by increased ROS levels, leading to mitochondrial dysfunction and cytochrome c release. The decline in mitochondrial MMP and activation of caspases further confirm apoptosis via the intrinsic pathway. These findings align with recent studies on *Chionanthus* species, reinforcing their potential as therapeutic agents.

The study also highlights the ability of *Chionanthus virginica* Q elicit selective cytotoxic responses in HepG2 cells through exposure-dependent oxidative stress and mitochondrial disruption, suggesting its potential application in cancer treatment. Future studies should focus on isolating bioactive compounds and exploring in vivo efficacy and safety to validate its therapeutic potential further.

Acknowledgement

The authors thank Vinayaka Mission's Research Foundation (VMRF) for financial support, Vinayaka Mission's Homoeopathic Medical College and Hospital, and Alpha Omega Research Foundation, Salem, Tamil Nadu, India for research facilities.

Financial support

This study was financially supported by VMRF under the Seed Money Grant (VMRF/Seed Money/AY 2023-24/VMHMCH/2). The funding covered experimental materials, cell culture maintenance, and assay-related expenses. The funder had no role in the study design, data collection, analysis, or interpretation of results.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

Author contribution

Arun R. Nair: Concepts, Definition of Intellectual Content, Literature Search, Experimental Studies, Data Acquisition, Manuscript Preparation, Manuscript Editing, Manuscript Review.

Venkatesan Hariram: Concepts, Definition of Intellectual Content, Experimental Studies, Data Analysis, Statistical Analysis, Manuscript Preparation, Guarantor.

Vettrivel Arul: Design, Literature Search, Experimental Studies, Data Acquisition, Data Analysis, Statistical Analysis, Manuscript Preparation, Manuscript Editing, Manuscript Review.

References

1. Shen C, Jiang X, Li M, *et al*. Hepatitis virus and hepatocellular carcinoma: Recent advances. *Cancers (Basel)*. 2023;15(2):533. doi: 10.3390/cancers15020533.
2. Devarbhavi H, Asrani SK, Arab JP, *et al*. Global burden of liver disease: 2023 update. *J Hepatol*. 2023;79(2):516–37.
3. Ferrari AJ, Santomauro DF, Aali A, *et al*. Global incidence, prevalence, years lived with disability (YLDs), disability-adjusted life-years (DALYs), and healthy life expectancy (HALE) for 371 diseases and injuries in 204 countries and territories and 811 subnational locations, 1990–2021: a systematic analysis for the Global Burden of Disease Study 2021. *The Lancet*. 2024;403(10440):2133–61.
4. Almeida PH, Matielo CEL, Curvelo LA, *et al*. Update on the management and treatment of viral hepatitis. *World J Gastroenterol*. 2021;27(23):3249–61.
5. Yang YQ, Wen ZY, Liu XY, *et al*. Current status and prospect of treatments for recurrent hepatocellular carcinoma. *World J Hepatol*. 2023;15(2):129–50.
6. Debela DT, Muzazu SG, Heraro KD, *et al*. New approaches and procedures for cancer treatment: Current perspectives. *SAGE Open Med*. 2021;9:20503121211034366. doi: 10.1177/20503121241288169.
7. Asma ST, Acaroz U, Imre K, *et al*. Natural products/bioactive compounds as a source of anticancer drugs. *Cancers (Basel)*. 2022;14(24):6203. doi:10.3390/cancers14246203.
8. Chihomvu P, Ganesan A, Gibbons S, *et al*. Phytochemicals in drug discovery—A confluence of tradition and innovation. *Int J Mol Sci*. 2024;25(16):8792. doi:10.3390/ijms25168792.
9. Chunarkar PP, Kaleem M, Mishra R, *et al*. Anticancer drug discovery based on natural products: From computational approaches to clinical studies. *Biomedicines*. 2024;12(1):201. doi: 10.3390/biomedicines12010201.
10. Mihai RA, Espinoza Caiza IA, Melo Heras EJ, *et al*. Comparative assessment of antioxidant activity and functional components of chionanthus virginicus and chionanthus pubescens from the andean region of Ecuador. *Pharmaceutics*. 2023;15(6):1676. doi:10.3390/pharmaceutics15061676.
11. Boyer L, Baghdikian B, Bun SS, *et al*. Chionanthus virginicus L.: phytochemical analysis and quality control of herbal drug and herbal preparations. *Nat Prod Commun*. 2011;6(6):753–8.
12. Murphy R. Lotus Materia Medica. 3rd ed. B. Jain Publisher's (P)Ltd. 2018.
13. Adharshna SP, Arul V. An in vitro comparative assessment of antidiabetic efficacy: *Abroma augusta* Q, *Gymnema sylvestre* Q, *Cephalandra indica* Q, *Momordica charantia* Q, *Syzygium jambolanum* Q in comparison to *Metformin*. *Indian J Res Homoeopathy*. 2026;20(1):45–59.
14. Bhargaw HN, Sharma M, Srivastava AK, *et al*. Unraveling the low-frequency triggered electromagnetic signatures in potentized homoeopathic medicine. *Mater. Sci. Eng. B*. 2023;292:116365. doi: 10.1016/j.mseb.2023.116365.
15. Arul V, Kandasamy R, Adharshna SP. Antioxidant and cytotoxic potential of potentized preparation of Cordyceps sinensis in vitro in cancer cell lines. *Indian J Res Homoeopathy*. 2026;20(1):45–59.
16. Blidisel A, Marcovici I, Coricovac D, *et al*. Experimental models of hepatocellular carcinoma—A preclinical perspective. *Cancers (Basel)*. 2021;13(15):3651. doi:10.3390/cancers13153651.
17. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. Sci. Technol.* 2004;26(2):211–9.
18. Repetto G, Peso A, Zurita JL. Neutral red uptake assay for the estimation of cell viability/cytotoxicity. *Nat Protoc*. 2008;3(7):1125–31.
19. Sinjorgo KM, Durak I, Dekker HL, *et al*. Bovine cytochrome c oxidases, purified from heart, skeletal muscle, liver and kidney, differ in the small subunits but show the same reaction kinetics with cytochrome c. *Biochim Biophys Acta*. 1987;893(2):251–8.
20. Jeon JS, Kwon S, Ban K, *et al*. Regulation of the intracellular ROS level is critical for the antiproliferative effect of quercetin in the hepatocellular carcinoma cell line HepG2. *Nutr Cancer*. 2019;71(5):861–9.
21. Sakamuru S, Attene-Ramos MS, Xia M. Mitochondrial membrane potential assay. *Methods Mol Biol*. 2016;1473:17–22.
22. Kntayya SB, Ibrahim MD, Mohd Ain N, *et al*. Induction of apoptosis and cytotoxicity by isothiocyanate sulforaphene in human hepatocarcinoma HepG2 cells. *Nutrients*. 2018;10(6):718. doi:10.3390/nu10060718.
23. Kwak JH, Kang MW, Roh JH, *et al*. Cytotoxic phenolic compounds from Chionanthus retusus. *Arch Pharm Res*. 2009;32(12):1681–7.
24. Li Y, Zhang H, Yu C, *et al*. New insights into mitochondria in health and diseases. *Int J Mol Sci*. 2024;25(18):9975. doi:10.3390/ijms25189975.
25. Gülçin İ, Elias R, Gepdiremen A, *et al*. Antioxidant activity of lignans from fringe tree (Chionanthus virginicus L.). *Eur Food Res Technol*. 2006;223(6):759–67.
26. Cecerska-Heryć E, Wiśniewska Z, Serwin N, *et al*. Can compounds of natural origin be important in chemoprevention? anticancer properties of quercetin, resveratrol, and curcumin—A comprehensive review. *Int J Mol Sci*. 2024;25(8):4505. doi: 10.3390/ijms25084505.
27. Zong Y, Li H, Liao P, *et al*. Mitochondrial dysfunction: mechanisms and advances in therapy. *Signal Transduct Target Ther*. 2024;9(1):1–29.

Induction de l'apoptose par les mitochondries via *Chionanthus virginica* Q : une approche cytotoxique et induisant un stress oxydatif dans les cellules de carcinome hépatocellulaire HepG2

Contexte : Le carcinome hépatocellulaire (CHC) est l'une des principales causes de décès par cancer dans le monde. Les options thérapeutiques actuelles sont limitées par le diagnostic tardif, les récidives et la toxicité. Les traitements alternatifs issus de plantes médicinales suscitent un intérêt croissant en raison de leurs propriétés anticancéreuses potentielles. *Chionanthus virginica*, traditionnellement utilisé pour les troubles hépatiques, contient des composés bioactifs aux effets hépatoprotecteurs et antioxydants. Cependant, son potentiel anticancéreux reste largement inexploré**. Objectif :** Cette étude évalue les effets antioxydants, cytotoxiques et apoptotiques de la teinture mère (Q) et de la teinture mère (12C) de *Chionanthus virginica* sur les cellules cancéreuses hépatiques HepG2 afin de déterminer son efficacité en tant qu'agent anticancéreux naturel. **Matériels et méthodes :** Des cellules HepG2 ont été traitées avec *Chionanthus virginica* Q et 12C à différents niveaux d'exposition (50 à 1 000 µL/mL ; équivalent d'extrait pour Q et équivalent de volume pour 12C). L'activité antioxydante a été déterminée par le test DPPH, tandis que les tests MTT et LDH ont évalué la cytotoxicité. Le dysfonctionnement mitochondrial a été analysé par les tests de potentiel de membrane mitochondrial (MMP) et de libération du cytochrome c, la production d'espèces réactives de l'oxygène (ROS) a été mesurée par fluorescence DCFH-DA et l'activité des caspases a été quantifiée par un test colorimétrique. **Résultats :** *Chionanthus virginica* Q a présenté la plus forte activité antioxydante (CI50 = 101,49 µg/mL) et la cytotoxicité la plus élevée (CTC50 = 330,35 µg/mL), tandis que la réponse observée avec *Chionanthus virginica* 12C n'a été interprétée que comme un effet biologique dans des conditions d'exposition équivalentes en volume. Des analyses de variance à un et deux facteurs (ANOVA) ont été réalisées pour évaluer la significativité statistique. La libération de LDH, la translocation du cytochrome c et les niveaux de ROS ont augmenté significativement ($p < 0{,}001$), induisant une dépolarisation mitochondriale et l'activation des caspases, confirmant ainsi l'apoptose. **Conclusion :** Cette étude démontre que *Chionanthus virginica* Q induit l'apoptose dans les cellules HepG2 via un stress oxydatif et un dysfonctionnement mitochondrial, ce qui confirme son potentiel en tant qu'agent anticancéreux naturel. Des études complémentaires devraient se concentrer sur l'isolement des composés bioactifs et la validation in vivo.

Mitochondrien-vermittelte Apoptoseinduktion durch *Chionanthus virginica* Q: Ein zytotoxischer und oxidativer Stress-getriebener Ansatz in HepG2-Hepatozellulärkarzinomzellen

Hintergrund: Das hepatozelluläre Karzinom (HCC) ist weltweit eine der häufigsten krebsbedingten Todesursachen. Die derzeitigen Therapieoptionen sind durch späte Diagnose, Rezidive und Toxizität begrenzt. Alternative Behandlungen mit Heilpflanzen gewinnen aufgrund ihrer potenziellen Antikrebseigenschaften an Bedeutung. *Chionanthus virginica*, traditionell bei Lebererkrankungen eingesetzt, enthält bioaktive Verbindungen mit leberschützenden und antioxidativen Wirkungen. Sein Antikrebspotenzial ist jedoch weitgehend unerforscht. **Ziel:** Diese Studie untersucht die antioxidativen, zytotoxischen und apoptotischen Wirkungen von *Chionanthus virginica* in Urtinktur (Q) und potenzierter Form (12C) auf HepG2-Leberkrebszellen, um seine Wirksamkeit als natürliches Antikrebsmittel zu bestimmen. **Material und Methoden:** HepG2-Zellen wurden mit *Chionanthus virginica* Q und 12C in definierten Konzentrationen (50–1000 µL/mL; Extraktäquivalent für Q und Volumenäquivalent für 12C) behandelt. Die antioxidative Aktivität wurde mittels DPPH-Assay bestimmt, während die Zytotoxizität mittels MTT- und LDH-Assays beurteilt wurde. Mitochondriale Dysfunktion wurde mittels MMP- und Cytochrom-c-Freisetzungs-Assays analysiert, die ROS-Bildung mittels DCFH-DA-Fluoreszenz gemessen und die Caspase-Aktivität mittels eines kolorimetrischen Assays quantifiziert. **Ergebnisse:** *Chionanthus virginica* Q zeigte die stärkste antioxidative Aktivität (IC50 = 101,49 µg/mL) und die höchste Zytotoxizität (CTC50 = 330,35 µg/mL), während die mit *Chionanthus virginica* 12C beobachtete Reaktion nur unter Volumenäquivalentbedingungen als biologischer Effekt interpretiert werden konnte. Zur Bestimmung der statistischen Signifikanz wurden eine ein- und eine zweifaktorielle Varianzanalyse durchgeführt. Die LDH-Freisetzung, die Cytochrom-c-Translokation und die ROS-Konzentrationen stiegen signifikant an ($p < 0{,}001$), was zu einer mitochondrialen Depolarisation und Caspase-Aktivierung führte und somit die Apoptose bestätigte. **Schlussfolgerung:** Diese Studie zeigt, dass *Chionanthus virginica* Q in HepG2-Zellen über oxidativen Stress und mitochondriale Dysfunktion Apoptose induziert und damit sein Potenzial als natürliches Antikrebsmittel unterstreicht. Weitere Studien sollten sich auf die Isolierung bioaktiver Verbindungen und deren In-vivo-Validierung konzentrieren.

_चियोनैंथस वर्जिनिका_ Q माइटोकॉन्ड्रिया जनित द्वारा अपोप्टोटिक प्रेरण: हेपजी2 हेपेटोसेल्यूलर कार्सिनोमा कोशिकाओं में एक साइटोटॉक्सिक और ऑक्सीडेटिव तनाव आधारित अधयन्न

पृष्ठभूमि: हेपेटोसेल्यूलर कार्सिनोमा (एचसीसी) विश्व स्तर पर कैंसर से होने वाली मौतों का एक प्रमुख कारण है, जहाँ वर्तमान चिकित्सीय विकल्प देर से पता चलने, पुनरावृत्ति और विषाक्तता के कारण सीमित हैं। औषधीय पौधों से प्राप्त वैकल्पिक उपचार अपने संभावित कैंसर-रोधी गुणों के कारण ध्यान आकर्षित कर रहे हैं। *चियोनैंथस वर्जिनिका*, जिसका पारंपरिक रूप से यकृत विकारों के लिए उपयोग किया जाता है, में यकृत-सुरक्षात्मक और एंटीऑक्सीडेंट प्रभाव वाले जैव-सक्रिय यौगिक पाए जाते हैं। हालांकि, इसकी कैंसर-रोधी क्षमता का अभी तक पूरी तरह से पता नहीं लगाया गया है। **उद्देश्य:** यह अध्ययन *चियोनैंथस वर्जिनिका* Q एवं शक्तिकृत(12C) रूपों के हेपजी 2 यकृत कैंसर कोशिकाओं पर एंटीऑक्सीडेंट, साइटोटॉक्सिक और अपोप्टोटिक प्रभावों का मूल्यांकन करना है, ताकि एक प्राकृतिक कैंसर-रोधी एजेंट के रूप में इसकी प्रभावकारिता का निर्धारण किया जा सके। **सामग्री और विधियाँ:** हेपजी2 कोशिकाओं को *चियोनैंथस वर्जिनिका* Q और 12सी के साथ परिभाषित एक्सपोज़र स्तरों (50-1000 μL/mL; Q के लिए अर्क-समतुल्य और 12सी के लिए आयतन-समतुल्य) पर उपचारित किया गया। एंटीऑक्सीडेंट गतिविधि का निर्धारण DPPH परख का उपयोग करके किया गया, जबकि MTT और LDH परख द्वारा साइटोटॉक्सिसिटी का आकलन किया गया। माइटोकॉन्ड्रियल शिथिलता का विश्लेषण MMP और साइटोक्रोम सी रिलीज परख के माध्यम से किया गया, ROS उत्पादन को DCFH-DA फ्लोरेसेंस द्वारा मापा गया, और कैस्पेस गतिविधि को एक कोलोरिमीट्रिक असे का उपयोग करके निर्धारित किया गया। **परिणाम:** *चियोनैंथस वर्जिनिका* Q ने सर्वाधिक एंटीऑक्सीडेंट गतिविधि (IC50 = 101.49 μg/mL) और उच्च साइटोटॉक्सिसिटी (CTC50 = 330.35 μg/mL) प्रदर्शित की, जबकि *चियोनैंथस वर्जिनिका* 12सी के साथ देखी गई प्रतिक्रिया को केवल आयतन-समतुल्य एक्सपोज़र स्थितियों के तहत एक जैविक प्रभाव के रूप में व्याख्यायित किया गया। सांख्यिकीय सार्थकता के लिए वन-वे और टू-वे एनोवा विश्लेषण किया गया। एलडीएच रिलीज, साइटोक्रोम सी ट्रांसलोकेशन और ROS स्तरों में उल्लेखनीय वृद्धि हुई ($p < 0.001$), जिससे माइटोकॉन्ड्रियल डीपोलराइजेशन और कैस्पेस सक्रियण हुआ, जो एपोप्टोसिस की पुष्टि करता है। *निष्कर्ष:* यह अध्ययन दर्शाता है कि *चियोनैंथस वर्जिनिका* Q ऑक्सीडेटिव तनाव और माइटोकॉन्ड्रियल शिथिलता के माध्यम से हेपजी2 कोशिकाओं में एपोप्टोसिस प्रेरित करता है, जो एक प्राकृतिक कैंसररोधी एजेंट के रूप में इसकी क्षमता का समर्थन करता है। आगे के अध्ययनों में जैवसक्रिय यौगिकों के पृथक्करण और इन विवो सत्यापन पर ध्यान केंद्रित किया जाना चाहिए।

Inducción apoptótica mediada por mitocondrias mediante *Chionanthus virginica* Q: Un enfoque citotóxico y basado en el estrés oxidativo en células de carcinoma hepatocelular HepG2

Antecedentes: El carcinoma hepatocelular (CHC) es una de las principales causas de muerte por cáncer en todo el mundo, y las opciones terapéuticas actuales se ven limitadas por la detección tardía, la recurrencia y la toxicidad. Los tratamientos alternativos derivados de plantas medicinales están ganando interés debido a sus potenciales propiedades anticancerígenas. *Chionanthus virginica*, tradicionalmente utilizada para trastornos hepáticos, contiene compuestos bioactivos con efectos hepatoprotectores y antioxidantes. Sin embargo, su potencial anticancerígeno aún no se ha explorado en profundidad. **Objetivo:** Este estudio evalúa los efectos antioxidantes, citotóxicos y apoptóticos de *Chionanthus virginica* en tintura madre (Q) y en forma potenciada (12C) sobre células de carcinoma hepatocelular HepG2 para determinar su eficacia como agente anticancerígeno natural. **Materiales y métodos:** Las células HepG2 se trataron con *Chionanthus virginica* Q y 12C en niveles de exposición definidos (50-1000 μL/mL; equivalente de extracto para Q y equivalente de volumen para 12C). La actividad antioxidante se determinó mediante el ensayo DPPH, mientras que los ensayos MTT y LDH evaluaron la citotoxicidad. La disfunción mitocondrial se analizó mediante ensayos de MMP y liberación de citocromo c, la generación de ROS se midió mediante fluorescencia de DCFH-DA y la actividad de caspasa se cuantificó mediante un ensayo colorimétrico. **Resultados***:* *Chionanthus virginica* Q mostró la actividad antioxidante más fuerte (IC50 = 101,49 μg/mL) y mayor citotoxicidad (CTC50 = 330,35 μg/mL), mientras que la respuesta observada con *Chionanthus virginica* 12C se interpretó solo como un efecto biológico en condiciones de exposición equivalentes de volumen. Se realizaron análisis de varianza unidireccionales

y bidireccionales para determinar la significación estadística. La liberación de LDH, la translocación del citocromo c y los niveles de ROS aumentaron significativamente ($p < 0,001$), lo que provocó despolarización mitocondrial y activación de caspasas, confirmando la apoptosis. **Conclusión:** Este estudio demuestra que *Chionanthus virginica* Q induce apoptosis en células HepG2 mediante estrés oxidativo y disfunción mitocondrial, lo que respalda su potencial como agente anticancerígeno natural. Futuros estudios deberían centrarse en el aislamiento de compuestos bioactivos y su validación in vivo.

白花雪蓮（*Chionanthus virginica* Q）透過粒線體介導誘導HepG2肝癌細胞凋亡：一種細胞毒性和氧化壓力驅動的機制

背景：肝細胞癌（HCC）是全球癌症相關死亡的主要原因之一，目前的治療選擇受限於晚期發現、復發和毒性。源自藥用植物的替代療法因其潛在的抗癌特性而備受關注。白花雪蓮（*Chionanthus virginica*）傳統上用於治療肝臟疾病，含有具有保肝和抗氧化作用的生物活性化合物。然而，其抗癌潛力仍未被充分開發。**目的：**本研究評估白花雪蓮母酊劑（Q）和增效劑（¹²C）對HepG2肝癌細胞的抗氧化、細胞毒性和凋亡作用，以確定其作為天然抗癌劑的療效。**材料與方法：**將HepG2細胞分別以不同濃度的雪絨花萃取物Q和12C處理（濃度範圍為50-1000 µL/mL；Q為萃取物當量，12C為體積當量）。採用DPPH法測定抗氧化活性，MTT和LDH法評估細胞毒性。透過MMP和細胞色素c釋放試驗分析粒線體功能障礙，DCFH-DA螢光法測定ROS生成，並以比色法定量caspase活性。**結果：**雪絨花萃取物Q表現出最強的抗氧化活性（IC50 = 101.49 µg/mL）和較高的細胞毒性（CTC50 = 330.35 µg/mL），而雪絨花12C在體積當量條件下的活性僅被解釋為生物效應。採用單因子和雙因子變異數分析（ANOVA）進行統計學顯著性檢定。乳酸脫氫酶（LDH）釋放、細胞色素c轉位和活性氧（ROS）水平顯著升高（$p < 0.001$），導致粒線體去極化和caspase激活，證實了細胞凋亡。**結論：**本研究表明，白花雪蓮（*Chionanthus virginica* Q）透過氧化壓力和粒線體功能障礙誘導HepG2細胞凋亡，支持其作為天然抗癌劑的潛力。後續研究應著重於生物活性化合物的分離和體內驗證。