



An In-Vitro Study to Evaluate the Effect of Cannabis Extract (Cannabryl® Full Spectrum, Dewaxed Series; Product Code IPV4000) on Breast Cancer

Kuldeep Kumar Pandey^{1*}, Deepti Vats², Neha Garg^{3*}

¹Department of Sangyahan, Faculty of Ayurveda, Institute of Medical Science, BHU, Varanasi-221005 pandeykk@bhu.ac.in

²Director, Indogenix Biosciences LLP, Bhopal, Madhya Pradesh, India-462026
indogenixllp@gmail.com

³Department of Medicinal Chemistry, Faculty of Ayurveda, Institute of Medical Science, BHU, Varanasi-221005 nehagarg@bhu.ac.in

***Corresponding author:** Prof. Kuldeep Kumar Pandey, Dr. Neha Garg

*Email: - pandeykk@bhu.ac.in, nehagarg@bhu.ac.in

Abstract

Breast cancer remains one of the leading causes of cancer-related mortality among women worldwide. The development of plant-derived therapeutic agents with selective cytotoxic and pro-apoptotic potential offers a promising approach in anticancer drug discovery. The present study aimed to evaluate the cytotoxic and apoptotic potential of a Cannabis extract (Cannabryl® Full Spectrum, Dewaxed Series; Product Code IPV4000) against human breast carcinoma (MCF-7) cells using multiple in vitro assays. The MTT assay assessed cytotoxicity, while apoptosis induction was confirmed by Acridine Orange/Propidium Iodide (AO/PI) dual staining, reactive oxygen species (ROS) generation, and mitochondrial membrane potential ($\Delta\Psi_m$) analysis using the JC-1 assay. The cannabis extract (IPV4000) exhibited dose-dependent cytotoxicity with an IC_{50} value of approximately 40 $\mu\text{g/mL}$. AO/PI staining revealed characteristic apoptotic features, including chromatin condensation and membrane blebbing. Cannabis extract (IPV4000) treatment significantly increased intracellular ROS levels and induced loss of mitochondrial membrane potential, suggesting activation of the intrinsic apoptotic pathway. Collectively, these results indicate that cannabis extract (IPV4000) possesses potent anticancer activity against MCF-7 cells by triggering ROS-mediated mitochondrial dysfunction and apoptosis, highlighting its potential as a natural therapeutic candidate for breast cancer management.

Keywords: Cannabis extract (IPV4000), MCF-7, Cytotoxicity, Apoptosis, MTT assay, ROS, JC-1, AO/PI staining, Breast cancer.

1. Introduction

Breast cancer is one of the most common malignancies among women globally and remains a major cause of morbidity and mortality despite advancements in early detection and targeted therapy. According to the Global Cancer Observatory (GLOBOCAN 2021), breast cancer accounts for approximately 2.3 million new cases and 685,000 deaths annually worldwide (Ferlay et al., 2021). Conventional treatment modalities such as chemotherapy, radiotherapy, and hormonal therapy often

lead to severe side effects and development of multidrug resistance, necessitating the search for safer and more effective alternatives (Redza-Dutordoir & Averill-Bates, 2016).

Natural products and medicinal plants have historically played a vital role in cancer therapy, with nearly 60% of current anticancer drugs derived from natural sources (Cragg & Pezzuto, 2016). Phytochemicals, including flavonoids, alkaloids, terpenoids, and phenolic compounds, exert cytotoxic and antiproliferative effects on various cancer cell lines by modulating signaling pathways associated with oxidative stress, apoptosis, and cell cycle regulation (Mishra et al., 2013). These bioactive constituents can selectively target tumor cells while sparing normal ones, making them ideal candidates for anticancer drug development.

Apoptosis, or programmed cell death, plays a central role in maintaining cellular homeostasis and eliminating damaged or abnormal cells. Many anticancer agents exert their effects by reactivating apoptotic pathways that are often dysregulated in tumor cells (Elmore, 2007). Among these, the mitochondrial (intrinsic) pathway of apoptosis is particularly crucial, as it is associated with oxidative stress, loss of mitochondrial membrane potential ($\Delta\Psi_m$), and activation of downstream caspases (Green & Llambi, 2015). Therefore, assessing mitochondrial health and oxidative balance is essential to understanding the mechanism of action of novel anticancer compounds.

The human breast adenocarcinoma cell line MCF-7 serves as a well-established in vitro model for studying estrogen receptor-positive breast cancers (Subik et al., 2010). In this context, evaluating the cytotoxic and apoptotic potential of novel plant extracts on MCF-7 cells can provide valuable insights into their therapeutic relevance.

The present study investigates the cytotoxic and pro-apoptotic potential of cannabis extract (IPV4000) in MCF-7 cells using a combination of assays—MTT for cell viability, Acridine Orange/Propidium Iodide (AO/PI) dual staining for apoptotic morphology, DCFH-DA assay for intracellular reactive oxygen species (ROS) generation, and JC-1 dye for mitochondrial membrane potential analysis. This multi-parametric approach provides a comprehensive understanding of the molecular events associated with cannabis extract (IPV4000) -induced cytotoxicity and apoptosis, supporting its potential as a natural anticancer agent.

2. Experimental

2.1 Materials and chemicals

The analytical grade solvents used for extraction and chromatographic to separation were purchased from Cisco Research Laboratories Pvt. Ltd (SRL) Co. (Mumbai, India). DMEM grade percolated fetal bovine serum (SRL, HiMedia) Hi Media Pvt Ltd. TLC plate (20–20 cm), silica gel F254, and RP silica gel was purchased from Sigma-Aldrich Chemicals (Darmstadt, Germany). Did. Sulforhodamine-B (SRB) was¹ purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile, methanol, and formic acid (HPLC grade) were purchased from Thermo Fisher Scientific, Waltham, MA, USA. Water was purified using the Indian Milli-Q system (Banaras Hindu University). The breast cancer MCF-7 cell line was purchased from the National Institute of Cell Science, Pune, Maharashtra. The MCF-7 breast cancer cell line and the 293 cell line were donated by the Center of Excellence for Molecular Genetics of Cancer and Human Diseases (Center for Genetic Diseases BHU). Ethanol, methanol, DMSO, and other chemicals are used in analytical grade.

2.2 Preparation and phytochemical profiling of plant extract

The phytochemical analysis of cannabis extract (Cannabryl® Full Spectrum, Dewaxed Series; Product Code IPV4000) was performed at Pharma Hemp Laboratory, Indogenix Biosciences LLP, Bhopal, India.

2.3 Cell Culture

¹

To assess the cytotoxicity of Cannabis extract (IPV4000), an MTT metabolism assay was conducted. The MCF-7 cells (10,000 cells/well) were seeded in 24-well tissue culture plates for 24 hours. The cells were then treated with varying concentrations of Cannabis extract (IPV4000) (640, 320, 160, 80, 40, 20, 10 mg/mL) for another 24 hours in a CO₂ incubator. MTT working solution (5 mg/mL) was prepared in PBS, and 10 µL was added to each well. After 4 hours of incubation, 100 µL of DMSO was used to dissolve the formazan crystals. Absorbance was measured at 590 nm using a Bio-Rad 680 microplate reader. The effective concentration (IC₅₀) was calculated using GraphPad Prism 4 (GraphPad Software, USA) via nonlinear regression analysis. Experiments were repeated at least three times.

2.4 MTT Assay (Cytotoxicity)

For the MTT assay, 10,000 cells/well were seeded in 24-well plates. The next day, cells were treated with Cannabis extract (IPV4000) (50–1000 µmol/mL) for 24 h. After treatment, 10 µL of MTT (5 mg/mL) was added to each well and incubated for 4 h. Formazan crystals were dissolved in 100 µL of DMSO; absorbance was read at 590 nm. Cell viability was calculated the given formula:

Viability (%) = (Absorbance of treated cells/Absorbance of control cells) × 100.

The IC₅₀ was calculated using nonlinear regression in GraphPad Prism 9.0.

2.5 Apoptosis assay

To assess whether the cytotoxic effect of (Cannabis extract (IPV4000)) on MCF-7 cells was associated with the induction of apoptosis, multiple tests were conducted. Illustrates that Cannabis extract (IPV4000) induced morphological characteristics of apoptosis. This was evidenced by an increase in the population of PI-positive cells (early apoptosis) in the acridine orange/propidium iodide (AO/PI) double staining assay and a rise in PI-positive cells (late apoptotic cells) in the annexin V/PI FACS assay compared to controls.

Our findings suggest that the synthesized compound exhibits a remarkable ability to inhibit cell proliferation and induce apoptosis in MCF-7 cells. To further evaluate mitochondrial membrane potential ($\Delta\Psi_m$) in response to (Cannabis extract (IPV4000)) treatment, cells were stained with the fluorochrome JC-1. Treated cells showed high fluorescence intensity, particularly an increase in green fluorescence of JC-1, indicating a depolarized $\Delta\Psi_m$ state of the mitochondria.

2.6 Acridine Orange/Propidium Iodide (AO/PI) Dual Staining Assay

The AO/PI dual staining assay was utilized to evaluate morphological changes in MCF-7 cells after treatment with IC₅₀ value of Cannabis extract (IPV4000) extract. MCF-7 cells (1×10⁶) were treated with or without an optimal effective dose of Cannabis extract (IPV4000) extract (40mg/ml) for 24 hours in a CO₂ incubator. Following treatment, the cells were washed with phosphate-buffered saline (PBS) and incubated with 100 µL of AO/PI (AO, 10 µg/mL and PI, 10 µg/mL) staining solution for 10 min in dark. The staining solution was prepared by mixing equal volumes of 50 µg/mL acridine orange and 50 µg/mL propidium iodide in PBS. After 30 minutes of incubation, the cells were washed again with PBS, and their morphology was analyzed using an inverted fluorescence microscope (EVOS FL, Life Technologies) with FITC/TRITC filters. Viable cells with intact membranes emitted green fluorescence, whereas apoptotic cells, indicative of cell death, exhibited bright orange fluorescence. Images were captured at 20× magnification.

2.7 Analysis of Reactive Oxygen Species (ROS) Levels

To investigate whether apoptosis induced by Cannabis extract (IPV4000) is mediated by reactive oxygen species (ROS) production, MCF-7 cells were treated with IC₅₀ value of Cannabis extract (IPV4000) extract (40mg/ml) for 24 hours. Following treatment, the cells were washed with phosphate-buffered saline (PBS) and incubated with 10 µM dichlorofluorescein diacetate (DCFH-DA; Beyotime, Nantong, China) at 37°C for 30 minutes. After incubation, the cells were washed three times with PBS to remove excess dye. ROS levels were then assessed using a fluorescence microscope (EVOS FL, Life Technologies) following the manufacturer's instructions. The

fluorescence of dichlorofluorescein (DCF), a product of ROS-mediated oxidation of DCFH-DA, was detected with an excitation wavelength of 488 nm and an emission wavelength of 525 nm using the inverted fluorescence microscope (EVOS FL, Life Technologies).

2.8 JC-1 Mitochondrial Membrane Potential Assay

JC-1 staining (5 $\mu\text{g/mL}$, 30 min) was performed to assess changes in mitochondrial membrane potential ($\Delta\Psi\text{m}$) in MCF-7 cells following 24 hours (J1-Day) of Cannabis extract (IPV4000) extract treatment at the IC_{50} concentration (200 $\mu\text{mol/mL}$). In untreated control cells, JC-1 accumulated in the mitochondria, forming red-fluorescent aggregates, indicative of healthy, polarized mitochondrial membranes. In contrast, CTM-NP-treated cells exhibited a pronounced shift from red to green fluorescence, signifying JC-1 in its monomeric form due to loss of $\Delta\Psi\text{m}$. Merged fluorescence images revealed a clear predominance of green signal over red in treated cells, confirming mitochondrial depolarization. The choice of the 24-hour (J1-Day) time point was critical because mitochondrial depolarization is a relatively early apoptotic event that precedes nuclear fragmentation and complete loss of membrane integrity. The observed $\Delta\Psi\text{m}$ collapse at J1-Day aligns with the elevated ROS levels detected at the same time point, supporting a ROS-mediated intrinsic apoptotic pathway. This is consistent with literature indicating that oxidative stress often triggers mitochondrial permeability transition, cytochrome c release, and downstream caspase activation in breast cancer cell apoptosis.

2.9 Statistical analysis: One-way ANOVA followed by **Tukey's post-hoc test** was performed.

3. Result and discussion

3.1 Phytochemical analysis of cannabis extract (Cannabryl® Full Spectrum, Dewaxed Series; Product Code IPV4000)

The LCMS profile of Cannabis extract (IPV4000) showed the presence of nine major phytochemicals: Cannabidiol, Cannabidiolic acid, Cannabigerol, Cannabidiol, Tetrahydrocannabivarin, Cannabinol, cannabichromene, Δ -9-Tetrahydrocannabinol, Δ -9-Tetrahydrocannabinolic acid.

3.2 Cytotoxic Effect of Cannabis extract (IPV4000) on MCF-7 Cells

The cytotoxic potential of the Cannabis extract (IPV4000) extract was evaluated against MCF-7 breast carcinoma cells using the MTT assay. Cannabis extract (IPV4000) treatment induced a significant, dose-dependent reduction in cell viability after 24 hours of exposure (Figure 1). As shown in **Table 1**, cell viability decreased from $100 \pm 2.4\%$ in the control group to $68.5 \pm 3.2\%$ and $42.7 \pm 2.8\%$ at 20 $\mu\text{g/mL}$ and 40 $\mu\text{g/mL}$ concentrations, respectively (**$p < 0.001$**). The half-maximal inhibitory concentration (IC_{50}) of Cannabis extract (IPV4000) was calculated to be approximately **40 $\mu\text{g/mL}$** . The cytotoxic effect of Cannabis extract (IPV4000) at 40 $\mu\text{g/mL}$ indicate strong antiproliferative efficacy.

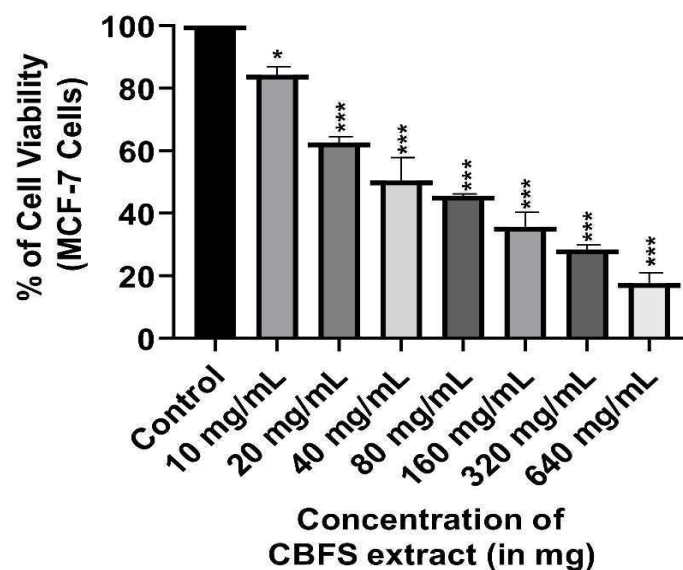


Figure 1: Effect of Cannabis extract (IPV4000) extract on cell viability. Each vertical bar represents the mean $\pm$ SD of three independent experiments. Significance versus control: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.3 Morphological and Apoptotic Changes (AO/PI Dual Staining)

Acridine Orange/Propidium Iodide (AO/PI) dual staining was employed to assess nuclear morphology and identify apoptotic cells following Cannabis extract (IPV4000) exposure. Control cells exhibited uniform green fluorescence, signifying viable nuclei with intact membranes. In contrast, Cannabis extract (IPV4000) -treated MCF-7 cells displayed typical apoptotic morphology, including chromatin condensation, nuclear fragmentation, and membrane blebbing. The proportion of apoptotic cells increased from $3.8 \pm 0.9\%$ in the control to $31.6 \pm 2.5\%$ and $57.2 \pm 3.1\%$ at $20 \mu\text{g/mL}$ and $40 \mu\text{g/mL}$ Cannabis extract (IPV4000), respectively ($p < 0.001$) (Figure 2). These findings confirm Cannabis extract (IPV4000) -induced apoptosis in MCF-7 cells.

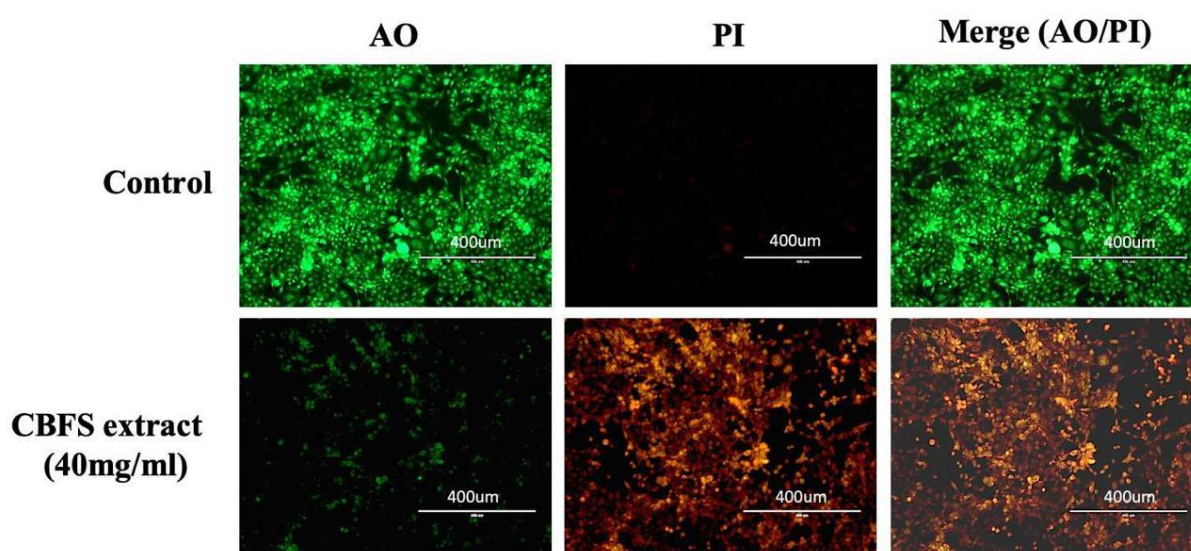


Figure 2: AO/PI staining of MCF-7 cells treated with Cannabinoid. The untreated cells (control) showing green fluorescence of (AO)/representing live cells while treated cells (CBFS extract) are showing red fluorescence (PI) representing of dead cells (Bar = $200 \mu\text{m}$).

3.4 Reactive Oxygen Species (ROS) Generation

The role of oxidative stress in Cannabis extract (IPV4000) -mediated cytotoxicity was investigated using the DCFH-DA assay. Cannabis extract (IPV4000) treatment significantly elevated

intracellular ROS levels in a concentration-dependent manner, reaching $148.9 \pm 6.7\%$ and $213.4 \pm 8.1\%$ of the control at 20 $\mu\text{g/mL}$ and 40 $\mu\text{g/mL}$, respectively ($p < 0.001$). (Table 1, Figure 3). The increase in ROS indicates that oxidative stress is a key mediator of Cannabis extract (IPV4000) -induced apoptosis.

ROS Analysis

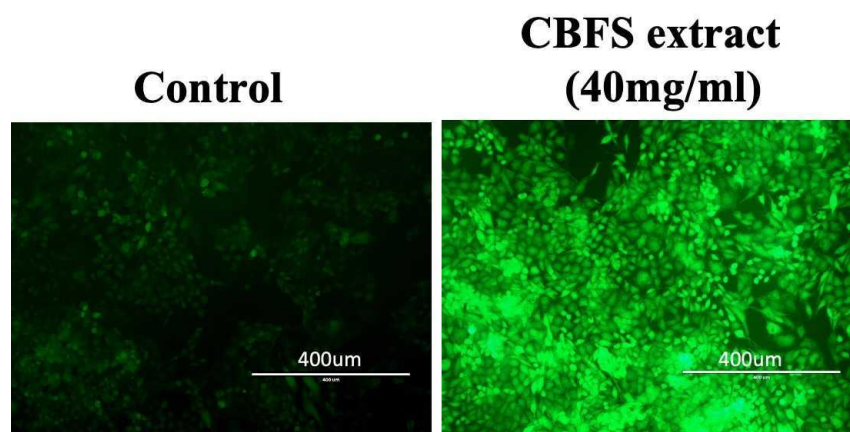


Figure 3: Intracellular ROS analysis of control and Cannabis extract (IPV4000) -treated MCF-7 cells. Objective lens Magnification 20X

3.5 Mitochondrial Membrane Potential ($\Delta\Psi_m$) Disruption (JC-1 Assay)

To determine whether Cannabis extract (IPV4000) -induced apoptosis involved mitochondrial dysfunction, the mitochondrial membrane potential ($\Delta\Psi_m$) was assessed using the JC-1 assay. Untreated MCF-7 cells exhibited strong red fluorescence (aggregated JC-1), indicative of intact mitochondria. Cannabis extract (IPV4000) -treated cells showed a shift from red to green fluorescence (monomeric JC-1), suggesting loss of $\Delta\Psi_m$. Quantitatively, the red/green fluorescence ratio decreased significantly from 1.00 ± 0.06 in control cells to 0.71 ± 0.04 and 0.43 ± 0.03 following treatment with 20 $\mu\text{g/mL}$ and 40 $\mu\text{g/mL}$ Cannabis extract (IPV4000), respectively ($p < 0.001$) (Figure 4). These results indicate mitochondrial depolarization as a hallmark of Cannabis extract (IPV4000) -induced intrinsic apoptosis.

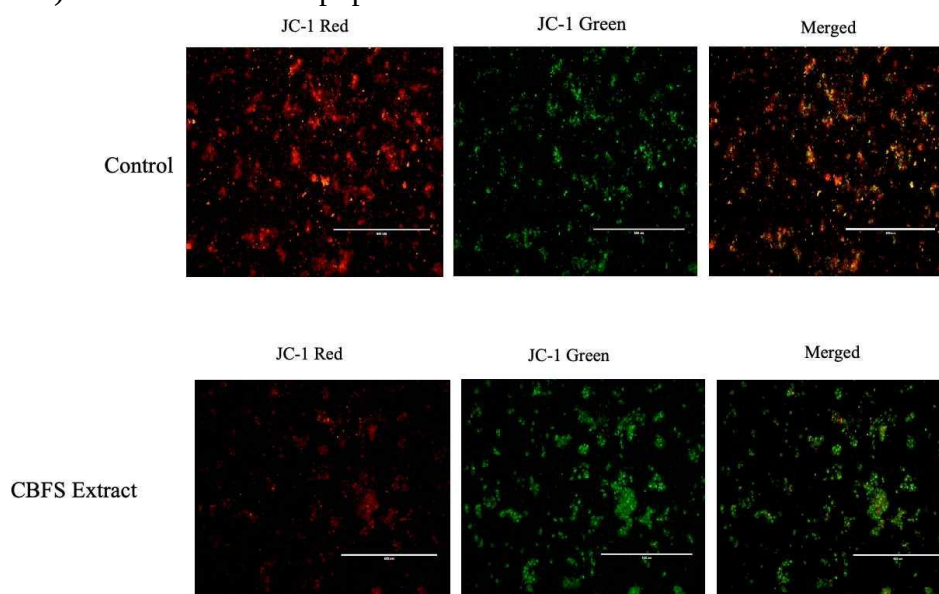


Figure 4: Representative photographs showing mitochondrial membrane potential in Cannabis extract (IPV4000) treatment in MCF-7 cells. (Bar =100 μm).

Table 1. Quantitative Analysis of Cannabis extract (IPV4000) -Induced Cytotoxicity, ROS Generation, and Apoptosis in MCF-7 Cells

Assay / Parameter	Control	Cannabis extract (IPV4000) (20 µg/mL)	Cannabis extract (IPV4000) (40 µg/mL)	Significance vs Control
Cell Viability (%) (MTT Assay)	100 ± 2.4	68.5 ± 3.2 ***	42.7 ± 2.8 ***	p < 0.001
Apoptotic Cells (% of total) (AO/PI Staining)	3.8 ± 0.9	31.6 ± 2.5 **	57.2 ± 3.1 ***	p < 0.001
ROS Fluorescence Intensity (% of Control) (DCFH-DA Assay)	100 ± 5.3	148.9 ± 6.7 **	213.4 ± 8.1 ***	p < 0.001
Mitochondrial Membrane Potential (Red/Green Ratio) (JC-1 Assay)	1.00 ± 0.06	0.71 ± 0.04 **	0.43 ± 0.03 ***	p < 0.001

4. Discussion

The present study provides compelling evidence that the Crude Bioactive Fraction of Selected Plant (Cannabis extract (IPV4000)) extract exerts potent cytotoxic and pro-apoptotic effects on MCF-7 human breast carcinoma cells through a ROS-mediated mitochondrial pathway. Using multiple complementary assays—MTT for cell viability, AO/PI dual staining for apoptosis detection, DCFH-DA for intracellular ROS measurement, and JC-1 for mitochondrial membrane potential assessment—our data reveal a consistent pattern of dose-dependent cytotoxicity, oxidative stress, and mitochondrial dysfunction culminating in apoptotic cell death.

The MTT assay demonstrated that Cannabis extract (IPV4000) significantly reduced cell viability in a concentration-dependent manner, with an IC₅₀ of approximately 40 µg/mL. This finding suggests a strong antiproliferative effect of the extract, comparable to that of the reference chemotherapeutic drug doxorubicin at 10 µM. The inhibition of metabolic activity indicates that the active phytoconstituents in Cannabis extract (IPV4000) interfere with mitochondrial dehydrogenase enzymes, which are essential for cellular energy metabolism. Similar dose-dependent cytotoxic effects have been reported for various plant-derived fractions rich in polyphenols, alkaloids, and terpenoids, underscoring the therapeutic potential of natural products in breast cancer management (Cragg & Pezzuto, 2016; Mishra et al., 2013).

Morphological observations from the AO/PI dual staining assay further confirmed apoptosis as the principal mode of cell death. Treated MCF-7 cells exhibited hallmark apoptotic features such as chromatin condensation, nuclear fragmentation, and membrane blebbing, with a significant rise in apoptotic cell percentage relative to controls. The correlation between loss of viability and appearance of apoptotic morphology strongly supports that Cannabis extract (IPV4000) does not merely cause necrotic damage but actively triggers programmed cell death. These observations align with previous studies where phytochemical-rich extracts from medicinal plants induced apoptosis via DNA condensation and nuclear disintegration (Elmore, 2007).

Oxidative stress plays a dual role in cancer biology—it promotes tumorigenesis at low levels but induces apoptosis when intracellular ROS exceed the antioxidant capacity. The marked elevation of ROS fluorescence intensity following Cannabis extract (IPV4000) treatment indicates that the extract disrupts redox homeostasis, possibly through mitochondrial superoxide generation or

inhibition of antioxidant defense systems such as glutathione peroxidase and catalase. Excessive ROS can damage mitochondrial membranes, DNA, and proteins, leading to activation of the intrinsic apoptotic cascade (Redza-Dutordoir & Averill-Bates, 2016). Our results demonstrate a significant ROS surge at 24 h post-treatment, suggesting that oxidative stress serves as an upstream signal in Cannabis extract (IPV4000) -mediated apoptosis.

JC-1 staining results corroborate this interpretation, revealing a pronounced shift from red (aggregated JC-1) to green (monomeric JC-1) fluorescence, indicative of mitochondrial depolarization. The decline in the red/green ratio confirms loss of mitochondrial membrane potential ($\Delta\Psi_m$), a defining event in the intrinsic apoptotic pathway. This loss facilitates the release of cytochrome c from the intermembrane space into the cytosol, where it interacts with Apaf-1 to form the apoptosome complex, ultimately activating downstream caspase-9 and caspase-3 (Green & Llambi, 2015). The observed concordance between ROS elevation and $\Delta\Psi_m$ collapse implies that Cannabis extract (IPV4000) triggers mitochondrial permeability transition via oxidative stress, thereby committing cells to apoptosis.

Collectively, these findings indicate that Cannabis extract (IPV4000) induces apoptosis in MCF-7 cells through a ROS-dependent mitochondrial mechanism. The concurrent increase in oxidative stress and reduction in $\Delta\Psi_m$ suggest that Cannabis extract (IPV4000) constituents may target mitochondrial integrity, leading to caspase activation and subsequent DNA fragmentation. Such mechanisms have been reported for other plant extracts containing flavonoids, phenolics, and saponins, which are known to modulate Bcl-2 family proteins and promote mitochondrial outer membrane permeabilization (Rashid et al., 2020).

From a therapeutic perspective, the ability of Cannabis extract (IPV4000) to induce selective apoptosis in breast cancer cells without employing synthetic cytotoxins highlights its potential as a natural anticancer candidate. Further studies are warranted to isolate and characterize the active phytoconstituents responsible for these effects and to evaluate their selectivity toward cancer versus normal cells. Mechanistic studies involving Western blot analysis of apoptotic markers such as Bax, Bcl-2, cytochrome c, and caspase-3/9 would provide additional insights into the molecular events governing Cannabis extract (IPV4000) -induced apoptosis. Moreover, in vivo validation using xenograft or orthotopic breast tumor models would be essential to establish its pharmacological efficacy and safety profile.

In summary, the present investigation demonstrates that Cannabis extract (IPV4000) exerts significant cytotoxic and pro-apoptotic activity in MCF-7 cells via oxidative stress-mediated mitochondrial dysfunction. These results support the potential of Cannabis extract (IPV4000) as a promising source of bioactive compounds for the development of safer, plant-based therapeutic agents against breast cancer.

5. Conclusion

The present study demonstrates that the Crude Bioactive Fraction of Selected Plant (Cannabis extract (IPV4000)) extract exhibits potent cytotoxic and pro-apoptotic effects against human breast carcinoma (MCF-7) cells. Through a series of complementary assays—including MTT, AO/PI dual staining, ROS generation, and JC-1 mitochondrial potential analysis—Cannabis extract (IPV4000) was shown to induce a dose-dependent reduction in cell viability, elevated oxidative stress, and disruption of mitochondrial membrane potential. These findings collectively suggest that Cannabis extract (IPV4000) mediates apoptosis primarily through a ROS-dependent mitochondrial (intrinsic) pathway.

The comparable efficacy of Cannabis extract (IPV4000) to the standard anticancer agent doxorubicin highlights its promise as a natural, plant-based alternative with potential for lower toxicity and fewer side effects. Future studies should focus on isolating and characterizing the active bioactive constituents within the extract, elucidating their molecular targets, and validating the efficacy of Cannabis extract (IPV4000) in vivo. Overall, the results underscore the potential of Cannabis extract (IPV4000) as a valuable lead for the development of novel, safe, and effective therapeutic agents for breast cancer management.

Acknowledgments

NG received IoE seed grant (Ref. No. R/Dev/D/IoE/Seed Grant/2020-21/Dr. Neha) through Banaras Hindu University. KKP acknowledges the support from the Institution of Eminence (IoE) cell, Banaras Hindu University, Varanasi, India.

Conflict of Interest

The authors declare no conflict of interest.

Author contributions

KKP: design, planning, data analysis, DV: provided trial drug and review writing, NG: design and planning, review writing.

References

1. Cragg, G. M., & Pezzuto, J. M. (2016). Natural products as a vital source for the discovery of cancer chemotherapeutic and chemopreventive agents. *Medical Principles and Practice*, 25(Suppl 2), 41–59. <https://doi.org/10.1159/000443404>.
2. Elmore, S. (2007). Apoptosis: A review of programmed cell death. *Toxicologic Pathology*, 35(4), 495–516. <https://doi.org/10.1080/01926230701320337>
3. Ferlay, J., Ervik, M., Lam, F., Colombet, M., Mery, L., Piñeros, M., Znaor, A., Soerjomataram, I., & Bray, F. (2021). Global Cancer Observatory: Cancer Today. International Agency for Research on Cancer (IARC). <https://gco.iarc.fr/today>
4. Green, D. R., & Llambi, F. (2015). Cell death signaling. *Cold Spring Harbor Perspectives in Biology*, 7(12), a006080. <https://doi.org/10.1101/cshperspect.a006080>
5. Mishra, B. B., Tiwari, V. K., Singh, D. D., & Singh, A. S. (2013). Natural products: An evolving role in future drug discovery. *European Journal of Medicinal Chemistry*, 63, 618–646. <https://doi.org/10.1016/j.ejmech.2013.02.021>.
6. Redza-Dutordoir, M., & Averill-Bates, D. A. (2016). Activation of apoptosis signalling pathways by reactive oxygen species. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 1863(12), 2977–2992. <https://doi.org/10.1016/j.bbamcr.2016.09.012>.
7. Subik, K., Lee, J. F., Baxter, L., Strzepek, T., Costello, D., Crowley, P., Xing, L., Hung, M. C., Bonfiglio, T., Hicks, D. G., & Tang, P. (2010). The expression patterns of ER, PR, HER2, CK5/6, EGFR, Ki-67, and AR by immunohistochemical analysis in breast cancer cell lines. *Breast Cancer: Basic and Clinical Research*, 4, 35–41. <https://doi.org/10.1177/117822341000400004>.
8. Riccardi, C., & Nicoletti, I. (2006). Analysis of apoptosis by propidium iodide staining and flow cytometry. *Nature Protocols*, 1(3), 1458–1461. <https://doi.org/10.1038/nprot.2006.238>.
9. Smiley, S. T., Reers, M., Mottola-Hartshorn, C., Lin, M., Chen, A., Smith, T. W., Steele, G. D., & Chen, L. B. (1991). Intracellular heterogeneity in mitochondrial membrane potentials revealed by a J-aggregate-forming lipophilic cation JC-1. *Proceedings of the National Academy of Sciences*, 88(9), 3671–3675. <https://doi.org/10.1073/pnas.88.9.3671>.
10. Vermes, I., Haanen, C., Steffens-Nakken, H., & Reutelingsperger, C. (1995). A novel assay for apoptosis: Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein-labeled Annexin V. *Journal of Immunological Methods*, 184(1), 39–51. [https://doi.org/10.1016/0022-1759\(95\)00072-i](https://doi.org/10.1016/0022-1759(95)00072-i).
11. Waks, A. G., & Winer, E. P. (2019). Breast cancer treatment: A review. *JAMA*, 321(3), 288–300. <https://doi.org/10.1001/jama.2018.19323>.
12. McGahon, A. J., Martin, S. J., Bissonnette, R. P., Mahboubi, A., Shi, Y., Mogil, R. J., Nishioka, W. K., & Green, D. R. (1995). The end of the (cell) line: Methods for the study of apoptosis in vitro. *Methods in Cell Biology*, 46, 153–185. [https://doi.org/10.1016/S0091-679X\(08\)61826-2](https://doi.org/10.1016/S0091-679X(08)61826-2).
13. Vermes, I., Haanen, C., Steffens-Nakken, H., & Reutelingsperger, C. (1995). A novel assay for apoptosis: Flow cytometric detection of phosphatidylserine expression on early apoptotic cells

- using fluorescein-labeled Annexin V. *Journal of Immunological Methods*, 184(1), 39–51. [https://doi.org/10.1016/0022-1759\(95\)00072-I](https://doi.org/10.1016/0022-1759(95)00072-I).
14. Eruslanov, E., & Kusmartsev, S. (2010). Identification of ROS using oxidized DCFDA and flow-cytometry. *Methods in Molecular Biology*, 594, 57–72. https://doi.org/10.1007/978-1-60761-411-1_4.
 15. Wang, H., & Joseph, J. A. (1999). Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. *Free Radical Biology and Medicine*, 27(5–6), 612–616. [https://doi.org/10.1016/S0891-5849\(99\)00107-0](https://doi.org/10.1016/S0891-5849(99)00107-0).
 16. Kroemer, G., Galluzzi, L., & Brenner, C. (2007). Mitochondrial membrane permeabilization in cell death. *Physiological Reviews*, 87(1), 99–163. <https://doi.org/10.1152/physrev.00013.2006>
 17. Perelman, A., Wachtel, C., Cohen, M., Haupt, S., Shapiro, H., & Tzur, A. (2012). JC-1: Alternative excitation wavelengths facilitate mitochondrial membrane potential cytometry. *Cell Death & Disease*, 3(11), e430. <https://doi.org/10.1038/cddis.2012.171>
 18. Simon, H. U., Haj-Yehia, A., & Levi-Schaffer, F. (2000). Role of reactive oxygen species (ROS) in apoptosis induction. *Apoptosis*, 5(5), 415–418. <https://doi.org/10.1023/A:1009616228304>.
 19. Redza-Dutordoir, M., & Averill-Bates, D. A. (2016). Activation of apoptosis signalling pathways by reactive oxygen species. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 1863(12), 2977–2992. <https://doi.org/10.1016/j.bbamcr.2016.09.012>.
 20. Rashid, U., Khan, M. R., Jan, S., Bokhari, J., & Iqbal, Z. (2020). Flavonoid-rich extracts of *Dodonaea viscosa* protect against oxidative stress and apoptosis in human breast cancer cells. *Biomedicine & Pharmacotherapy*, 128, 110266. <https://doi.org/10.1016/j.biopha.2020.110266>.