

Anticancer Effect of Mahanimbine on MCF-7 Breast Cancer Cells: Analysis of Cell Viability, Oxidative Stress, Mitochondrial Functioning and Nuclear Changes

Ashapura Sinha¹, Ahamad Faiz Khan², Farina Mujeeb^{1*}

^{1*}Department of Biosciences, Faculty of Science,

Integral University, Kursi Road, Lucknow, 226026, India.

Email: farinam@iul.ac.in*, ashapurnasinha10@gmail.com.

ORCID: 0000-0001-7973-6843*, 0009-0008-7514-8559.

²Department of Bioengineering, Faculty of Engineering and Information Technology,

Integral University, Kursi Road, Lucknow, 226026, India.

Email: faizkhan@iul.ac.in, 0000-0003-2135-3596.

ABSTRACT

Cancer continues to be one of the major public health concerns and encourages the development of new drugs having anticancer properties. One such compound that has received attention due to its pharmacological properties is Mahanimbine (MB), which is a carbazole alkaloid compound from *Murraya koenigii*. MB has been tested for its effects on the viability, mitochondrial membrane potential, ROS production and nucleus of MCF-7 cells. MCF-7 cells were exposed to various doses of MB and the toxicity of MB was assessed through MTT test. Changes in the mitochondrial membrane potential upon MB treatment were observed with the help of Rhodamine 123 staining. Intracellular production of ROS was measured by using fluorescent dye. Nuclear changes were determined through DAPI staining technique. MB exhibited dose-dependent inhibition of viability of MCF-7 cells with IC₅₀ value of 45.6 μM. Treatment with MB resulted in alteration of the Rhodamine 123 fluorescence staining due to the effect of the drug on the mitochondria's membrane potential. In addition, MB treatment led to the increase in intracellular ROS production compared to untreated cells. Chromatin condensation and nuclear fragmentation were observed during DAPI staining indicating nuclear changes characteristic of apoptotic cells. MB demonstrated cytotoxicity against MCF-7 breast cancer cells. Generation of ROS, depolarization of mitochondrial membrane and alteration in nuclear structure indicate that oxidative stress and mitochondria dysfunction contribute to the mechanism of MB-induced cell death. Therefore, it can be seen that there is a potential in MB as a possible anticancer drug against breast cancer.

Keywords: Mahanimbine; MCF-7; Breast cancer; IC₅₀; Rhodamine 123; Reactive oxygen species; Mitochondrial membrane potential; DAPI; Apoptosis.

How to cite this article: Ashapura Sinha¹ Ahamad Faiz Khan² Farina Mujeeb^{1*}. Anticancer Effect of Mahanimbine on MCF-7 Breast Cancer Cells: Analysis of Cell Viability, Oxidative Stress, Mitochondrial Functioning and Nuclear Changes. Int J Drug Deliv Technol. 2026;16(84s):756-761. DOI: 10.25258/ijddt.16.84s.83.

1. INTRODUCTION

Breast cancer is the most frequent malignant disease; it can be considered the public health issue due to significant contribution of this oncological disease to the incidence and mortality rates among women (Smolarz et al., 2022). It is a highly heterogeneous tumour which differs in its histological features, molecular alterations, receptor status, behaviour and response to therapy. According to certain characteristics at the molecular level, breast cancer is classified into several types like HER2-positive, hormone receptor-positive, and triple-negative breast cancer (Tsang & Gary, 2020). These variations affect the course and management of the disease. However, despite the significant advances in early detection of breast cancer, its diagnosis, surgical and nonsurgical treatments, resistance, failure, relapse and toxicity

remain important issues (Wang & Wu, 2023). As a result, search and investigation of novel bioactive substances with antitumor activity remains relevant in cancer studies (Asma et al., 2022). Natural products have made a significant contribution to the discovery of new therapies and remain a promising source of structurally diverse compounds which can serve for the development of new pharmaceuticals (Naeem et al., 2022). Natural compounds derived from plants are especially interesting because of their complex chemical structure including secondary metabolites. Such compounds can regulate various physiological processes that are responsible for the tumorigenesis including uncontrolled proliferation, oxidative stress, mitochondrial dysfunctions, cell cycle regulation, inflammation, autophagy and programmed cell death (Dehelean et al., 2021). Study of such compounds can be useful for the development of new approaches

for cancer treatment. *Murraya koenigii* (L.) Spreng., commonly called curry leaf is a plant from the family Rutaceae which is widely used because of its nutritional, traditional and medicinal value (Balakrishnan et al., 2020). Many bioactive substances including carbazole alkaloids have been identified in the course of phytochemical analysis of *M. koenigii* (Hussien et al., 2025). Several bioactivities of carbazole alkaloids isolated from *M. koenigii* have been found to include antioxidative, antimicrobial, anti-inflammatory, and antitumor activities. One of the compounds isolated from *M. koenigii* is MB – prenylated carbazole alkaloid which has received much attention owing to its pharmacological potential. MB was investigated for the influence of this compound on processes of cancer cell survival and proliferation (Bose et al., 2025). Important mechanism of action of antitumor drugs includes the disruption of cellular redox balance. Cancer cells usually have altered metabolic pathways and higher basal levels of reactive oxygen species (ROS) (Mishra et al., 2023). This makes them vulnerable to further elevations in oxidative stress (Venugopal et al., 2026). Elevated ROS levels result in oxidative damage to proteins, lipids, DNA, and other cellular structures and ultimately cell death. Mitochondria play an important role in this process because they are involved in cellular bioenergetics, ROS production and regulation of programmed cell death (Hobani, 2022). Altered mitochondrial membrane potential is a sign of mitochondrial dysfunction and can be followed by other events leading to cell death. MCF-7 cancer cell line is widely used in vitro model which helps to study the biology of breast cancer and antitumor effects of test compounds (Sibuh et al., 2021). Characteristic features and reproducibility of this cell line make it suitable for the study of treatment-induced changes in cell viability and cellular morphology (Ahmad et al., 2022). In the current study, cytotoxicity of MB was assessed by means of MTT test, which is based on measurement of changes in metabolic activity and cell viability. The IC₅₀ value represents a quantifiable indicator of the concentration that causes inhibition of 50% cell viability. In addition to cytotoxicity, assessment of mitochondrial dysfunction and oxidative stress can reveal possible mechanisms of mahanimbine-induced cellular effects. Staining with Rhodamine 123 was done to determine the alteration of mitochondrial membrane potential following treatment (Aniogo et al., 2023). The changes in the fluorescence intensity are an indicator of mitochondrial function and cellular bioenergetics. In addition, the generation of intracellular ROS was evaluated to reveal whether oxidative stress is involved in mahanimbine-induced cytotoxicity (Ullah et al., 2020). DAPI staining was used for visualization of the changes in the nucleus and identification of the changes such as chromatin condensation, nuclear fragmentation, etc. which can be associated with apoptosis (Moradipour et al., 2022). Thus, the purpose of this research is to evaluate the antitumor activity of

MB in MCF-7 breast carcinoma cells. This includes the assessment of the cytotoxic effect of MB via MTT analysis and calculation of IC₅₀ (45.6 µM). Also, alteration in the mitochondrial membrane potential was examined via Rhodamine 123 dye. The intracellular generation of ROS was detected using a fluorochrome sensitive to ROS, and the nuclear structure was evaluated with the help of DAPI staining (Betts, Z et al., 2023). All of these techniques were employed to assess the role of oxidative stress, mitochondrial dysfunction, and nuclear changes in the reduction of MCF-7 cell viability after MB administration. The results of the study will give more information about mahanimbine cellular effects and lay the foundation for further investigation of this natural carbazole alkaloid as antitumor agent.

2. METHODOLOGY

Chemicals

MB (mahanimbine) (chemical abstract services registry number: 21104-28-9) (Cayman Chemicals, USA); MCF-7 Cells purchased from National Centre of Cell Science (NCCS), Pune; MTT Assay Kit (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl tetrazolium bromide); Dimethyl Sulfoxide (DMSO) HIMEDIA; 4,6-Diamidino-2-phenylindole (DAPI) HIMEDIA; 2',7'-Dichlorodihydro-fluorescein diacetate (H2DCFDA kit) Invitrogen; Rhodamine 123 (Cayman Chemicals, USA), Gibco, The highest-quality Gibco RPMI 1640, PBS, as well as other solvents and chemicals were purchased for the experiment. Distilled water was used as an aqueous solution in the experiment. The buffers were prepared with the help of 0.2µm filter paper and were used at once.

Maintenance of Cell Culture

Human breast carcinoma cell line (MCF-7) cells were procured from NCCS (National Centre for Cell Science), Pune, India. MCF-7 cell line was maintained in RPMI-1640 medium containing 10% FBS (fetal bovine serum) and 1% antibiotics. Cultured cells were maintained in an incubator at 37°C temperature and 5% CO₂ atmosphere. Subculture was done twice in a week before reaching confluence of 80%.

MTT assay for Cell Viability Test

The cytotoxicity of Mahanimbine (MB) towards MCF-7 breast carcinoma cells was assessed by MTT test as described earlier with certain modifications (Manikyam et al., 2021). Moreover, The MCF-7 cells were grown at the density of 5×10^3 cells per well in 96-well plates and incubated in an incubator for 24 hours to allow cell adhesion. The MB was dissolved in DMSO and then diluted in different concentrations (3.5, 7, 15, 30, 60 and 120 µM). Then the cells were exposed to the appropriate concentration of the MB and kept in an incubator with 5% CO₂ at 37 °C for 24 hours. In case of the control group, untreated cells along with culture medium was included in the study. The wells were then treated with MB and MTT reagent (10 µL; 5 mg/mL)

was added and allowed to incubate for 4 hours at 37 °C to obtain violet coloured formazan crystals due to the metabolic reaction taking place within live cells. Later on, the culture medium was withdrawn from all the wells and the crystals were solubilized using 100 µL of DMSO per well(Alshehri et al., 2022).

Morphology of MB pretreated cells

Morphological alterations caused by the action of MB on MCF-7 cells were investigated using the method of phase-contrast microscopy. For this purpose, about 5×10^3 cells/well were plated in a 96-well plate and were incubated for overnight attachment. Afterwards, the cells were exposed to the above-mentioned concentrations of MB and further incubated for 24 hours in accordance with the standard cultivation conditions. Differences in morphology of MB treated and untreated control cells were assessed after the incubation period. Pictures were made in the relief phase mode on the FLoid Imaging Station at 20× magnification (Thermo Fisher Scientific, USA)(Marsico et al., 2021).

Identification of nuclear condensation

Analysis of Apoptosis Index was carried out qualitatively in the MCF-7 cell line as a result of the effect of MB on the nuclei stained with DAPI. Cells were cultured in 6×10^3 cells/mL density in 6-well plates and left overnight to grow. Thereafter, the cells were incubated with different concentrations of MB as stated above for 24 hours. The medium was then disposed, and the cells were fixed using a cold PBS solution and cold methanol for 10 minutes. The cells were permeabilized using 3% paraformaldehyde and 0.25% Triton X-100(Hasan et al., 2023). Alterations in nuclear structure including chromatin condensation and fragmentation were visualized in the blue fluorescence on FLoid Imaging Station (Thermo Fisher Scientific, USA) by excitation/emission wavelengths of 390/40 and 446/33, respectively).

Evaluation of ROS Generated by Mahanimbine in MCF-7 Cells

The production of reactive oxygen species by the cells in intracellular organelles stimulated with MB was quantified and determined with the help of application of 2',7'-dichlorodihydrofluorescein diacetate (DCFH-

DA)(Jomova et al., 2023). For fluorescent microscopy, MCF-7 cells were grown in 12-well plates by using 2×10^4 cells/well density. Thereafter, the cells were treated with MB at various concentrations for 24 hours. To observe the production of reactive oxygen species, media were removed from the cells, and then cells were incubated in 10 µM DCFH-DA at room temperature for 30 minutes in the dark condition. Subsequently, the cells were washed in PBS buffer, and then production of intracellular ROS was observed in the fluorescence channel of FLoid Imaging Station (ex: 482/18 nm; em: 532/59 nm; Thermo Fisher Scientific, USA).

Measurement of Mitochondrial Membrane Potential ($\Delta\Psi_m$) Assay

The alterations in $\Delta\Psi_m$ of MB-treated cells were studied using fluorescence staining with Rhodamine 123 dye. The cells were cultured in 24 well plates using culture medium with 1×10^5 cells per well. The cells were given time to adhere before adding an effective amount of MB and incubating for 24 hours. This was followed by staining of cells using 300 nM of rhodamine 123 stain for 30 minutes at room temperature (RT). The microphotographs cells were taken using green fluorescence of FLoid Imaging Station (Thermo Fisher Scientific, USA).

3. RESULTS AND DISCUSSIONS

MB cytotoxicity and anticancer activity

For studying the cytotoxic effect and anti-proliferation effect of MB on MCF-7 cells, an MTT assay was carried out after treating MCF-7 with various concentrations of MB (3.5, 7, 15, 30, 60 and 120 µM) for 24 hours. The MCF-7 cells that have been exposed to MB have showed a significant reduction in their cell viability as shown in Figure 1, which is $95.1 \pm 2.1\%$, $90.7 \pm 2.4\%$, $80.2 \pm 2.2\%$, $62.4 \pm 2.0\%$, $38.7 \pm 1.7\%$ and $19.8 \pm 1.2\%$ for different concentrations of 3.5, 7, 15, 30, 60 and 120 µM of MB, respectively (Figure 1, 2). The optical density of plate was measured in Thermo Fisher plate reader at 570 nm. Optical density was calculated for the percentage of cytotoxicity.

% Cell viability = (OD value for test/OD value for control) $\times$ 100

% cytotoxicity = 100 - % cell viability

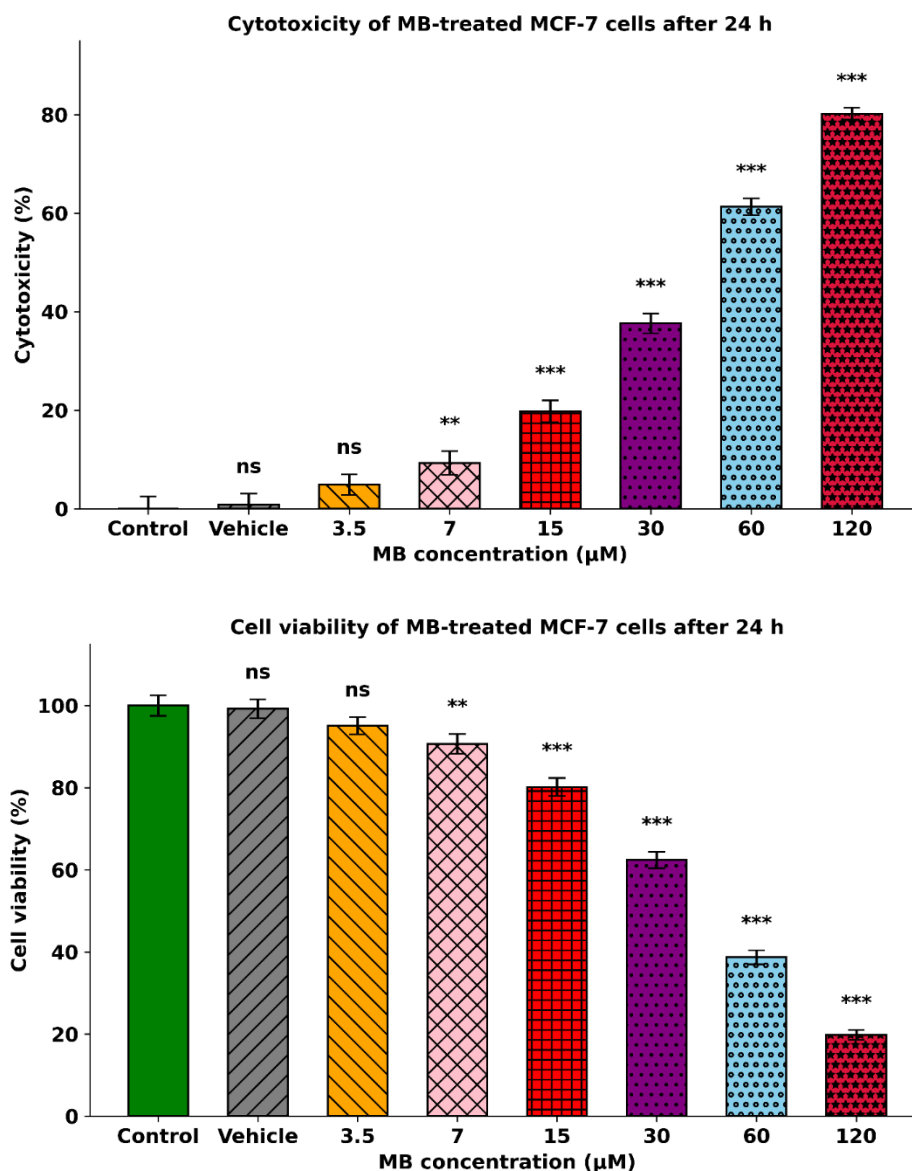


Figure 1: Anticancer assessment of Mahanimbine by MTT assay

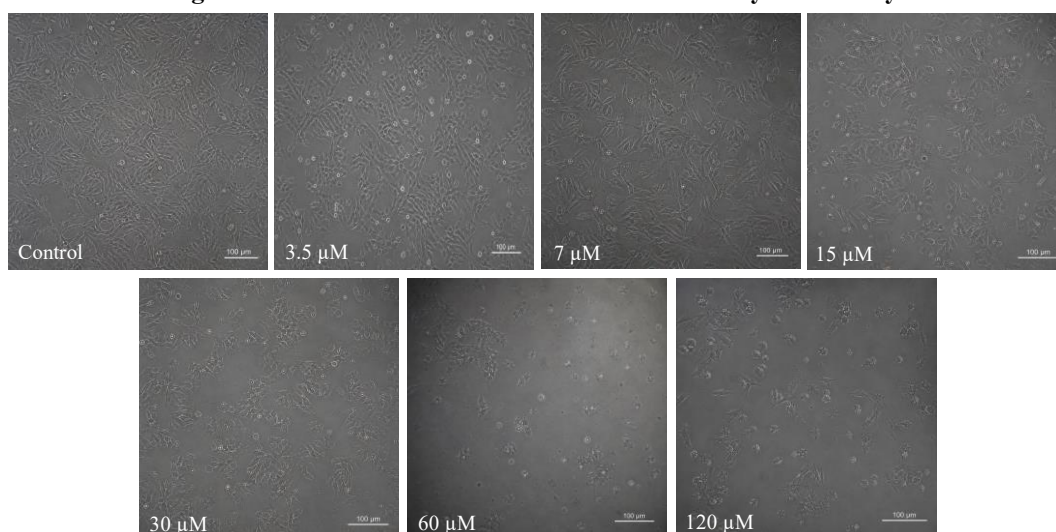


Figure 2. Mahanimbine-induced inverted phase contrast images demonstrating dose dependent morphological alterations in MCF-7 cells.
ligand: MCF-7 cell viability following treatment with mahanimbine (3.5–120 μM) for 24 h was
IJDDT Volume 16 Issue 1 2026

determined using the MTT assay. The IC_{50} of mahanimbine was calculated as 45.6 μM after 24 h of treatment. Values are presented as mean $\pm$ SD of three independent experiments, each performed in triplicate. Statistical significance was determined relative to the control group and is indicated as ns ($p > 0.05$), * $p < 0.05$, ** $p < 0.01$, and * $p < 0.001$.**

MTT assay

The cytotoxic effect of Mahanimbine on MCF-7 breast carcinoma cell line was measured by MTT test (Samanta et al., 2024). MTT assay is a colorimetric assay where the yellow dye, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is oxidized to a dark purple coloured product, formazan, by succinate dehydrogenase enzyme in living cells (Rego et al., 2026). The more viable the cells are, the more purple formazan is formed (0-120 $\mu g/mL$) and cell viability was assessed using the formation of formazan crystals. Mahanimbine demonstrated dose-dependent cytotoxicity on MCF-7 cells (Fig. 2). The IC_{50} of mahanimbine was calculated as 45.6 μM .

Assessment of mitochondrial membrane potential ($\Delta\Psi_m$)

The mitochondrial membrane potential ($\Delta\Psi_m$) exhibited a considerable decline upon treatment of the cells with mahanimbine for 24 hours compared to the untreated controls (Hu et al., 2023). It was shown that the fluorescence of rhodamine 123 was directly proportional to $\Delta\Psi_m$; the fluorescence was highest in the untreated control cells. After treatment with mahanimbine, there was a gradual decrease in the fluorescence value with increasing concentrations of the drug. These findings suggest that mahanimbine causes a concentration-dependent decrease in $\Delta\Psi_m$, indicating depolarization of mitochondrial membranes. This implies that mitochondria serve as the site for apoptosis induction.

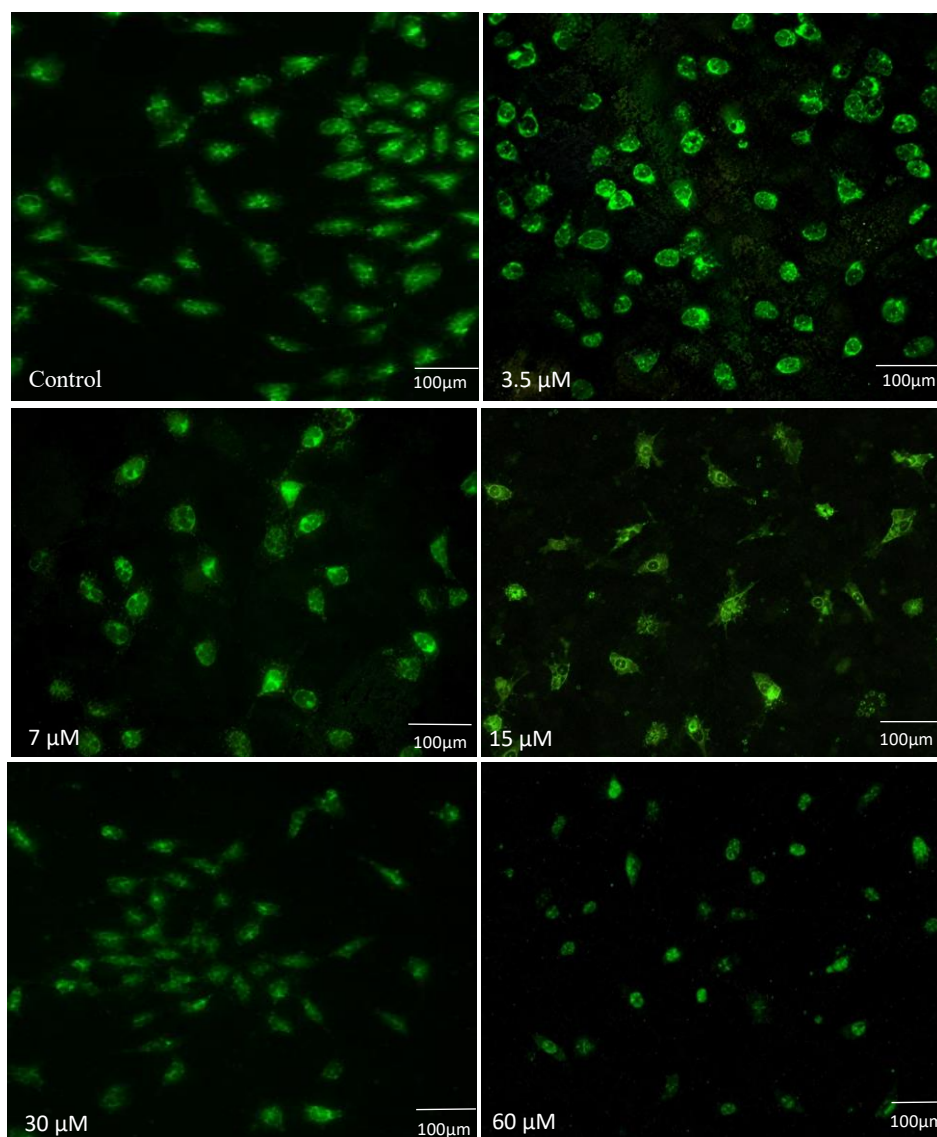


Figure 3. Rhodamine 123 fluorescence microscopy showing changes in mitochondrial membrane potential in MCF-7 cells following 24 h treatment with mahanimbine at 3.5, 7, 15, 30 and 60 μ M. Images were acquired at 20 $\times$ magnification; scale bar = 100 μ m.

Assessment of Nuclear Condensation

In MCF-7 cells, after 24 hours exposure to mahanimbine in different concentrations, showed significant nuclear condensation and fragmentation, while normal nuclear morphology was observed in

untreated control cells (Figure 4). There was also a concentration-dependent occurrence of the significant nuclear changes. The observed chromatin condensation and nuclear fragmentation are characteristics of apoptosis-associated nuclear changes.

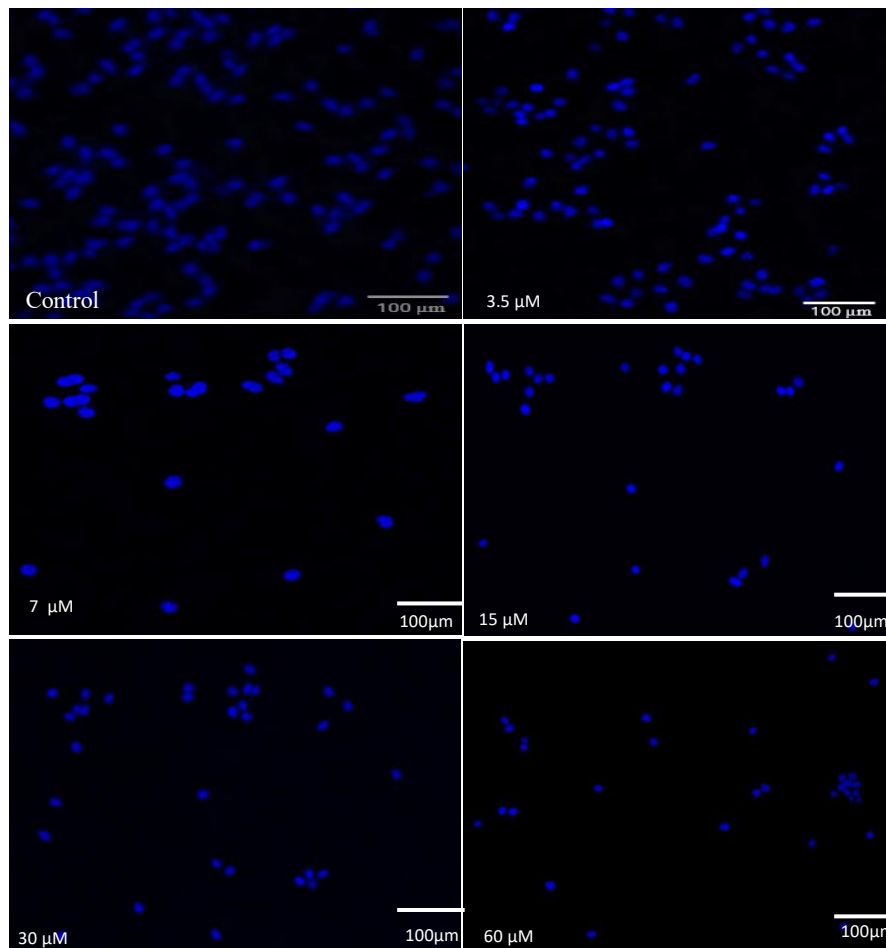


Figure 4: DAPI staining shows the nuclear Morphology of MCF-7 cells following 24 hours of Mahanimbine treatment at concentrations 3.5, 7, 15, 30, 60 μ M. The figures below represent morphological changes in the nucleus in a concentration-dependent manner (scale bar: 100 μ m; magnification: $\times 20$).

Assessment of ROS production

The level of ROS in MCF-7 cells was significantly increased after 24 hours exposure to different concentrations of Mahanimbine (Figure 5). DCF fluorescence was directly proportional to ROS levels in cells exposed to Mahanimbine. Untreated control cells did not have any detectable fluorescence, while there was a significant increase in fluorescence after

treatment with Mahanimbine. Moreover, ROS levels significantly increased with increasing concentrations of Mahanimbine from 3.5 μ M. Hence, Mahanimbine has been found to induce generation of ROS in a dose dependent manner in MCF-7 cells. Also, ROS production indicates oxidative stress induced by Mahanimbine and can be responsible for apoptosis induction in MCF-7 cells.

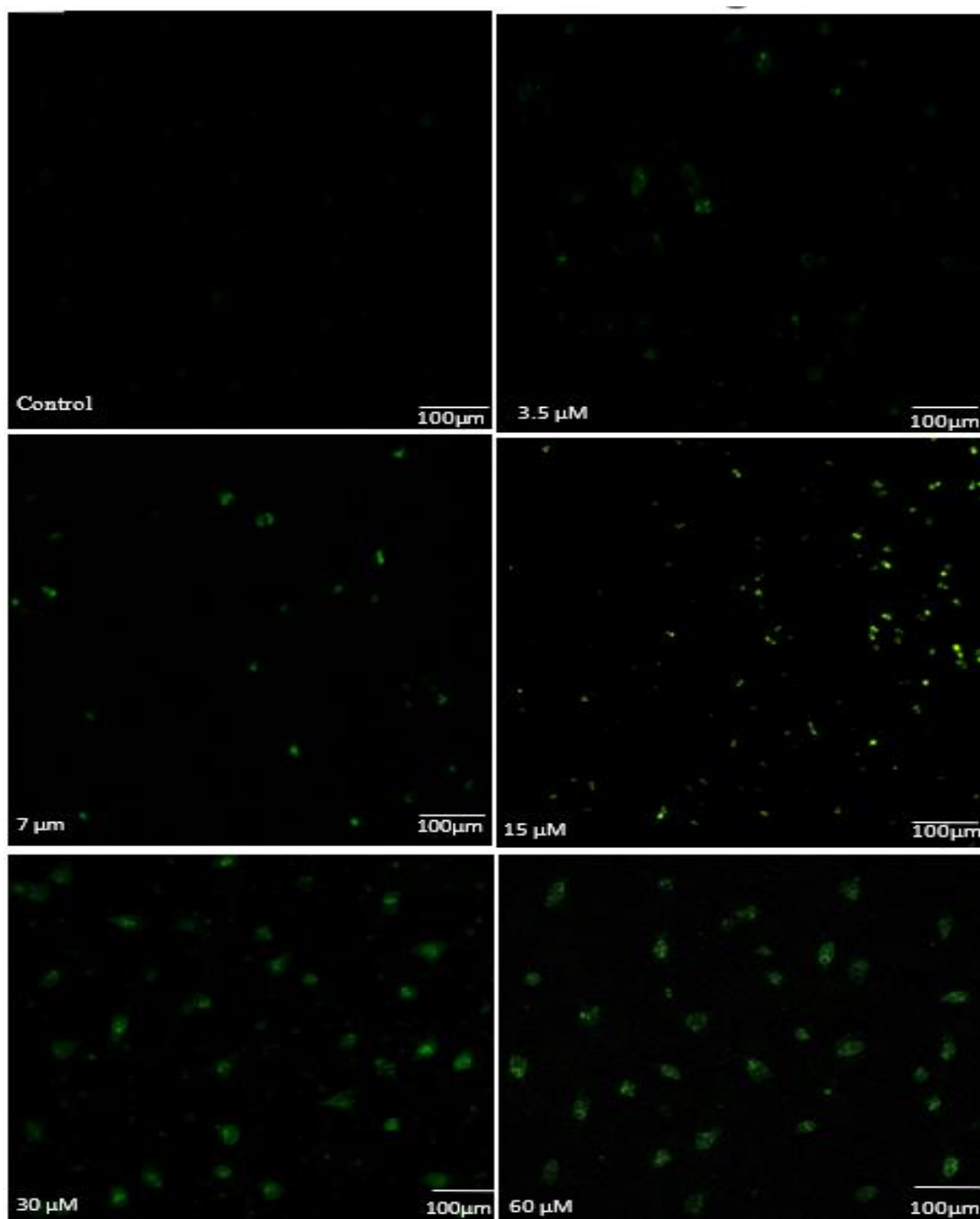


Figure 5: Qualitative analysis of ROS level in MCF-7 cells stained with H₂DCFDA with treatments of 3.5, 7, 15, 30, and 60 μM after 24 h using fluorescence microscopy. The images are representative of three independent experiments (scale bar: 100 μm; magnification: ×20). All of the images were taken by the same control picture.

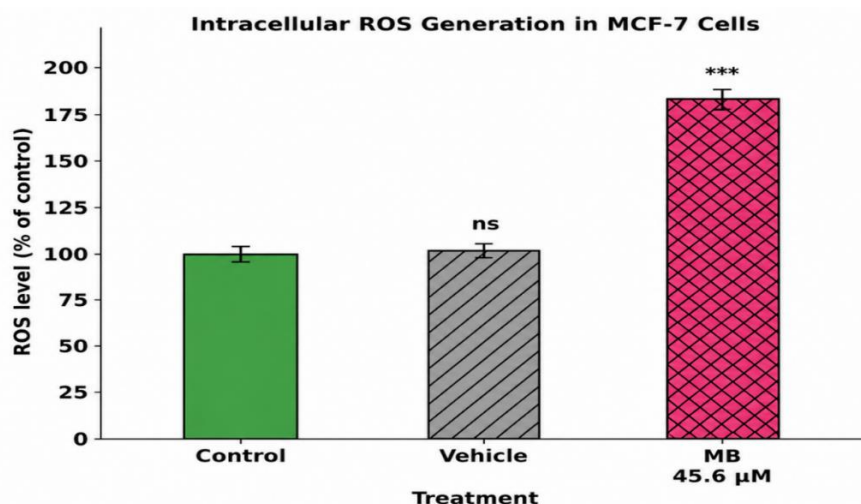


Figure 6: Graphical representation of the Intracellular ROS Generation in MCF-7 Cells

CONCLUSION

In conclusion, the present study demonstrates that mahanimbine exerts a cytotoxic effect against MCF-7 breast cancer cells and induces multiple cellular alterations associated with cellular stress and apoptosis. Phase-contrast microscopic observations showed noticeable morphological changes in mahanimbine-treated cells, including alterations in cell morphology and reduced cellular density compared with untreated control cells. Consistent with these observations, the MTT assay demonstrated a concentration-dependent reduction in cell viability following mahanimbine treatment, with an IC_{50} value of 45.6 μ M after 24 h of exposure. DAPI staining further revealed distinct nuclear alterations in mahanimbine-treated cells, including chromatin condensation and nuclear fragmentation, which are characteristic of apoptosis-associated nuclear changes. Assessment of mitochondrial membrane potential using Rhodamine-123 showed a decrease in mitochondrial membrane potential following mahanimbine treatment, suggesting impairment of mitochondrial function. In parallel, H_2DCFDA -based ROS analysis demonstrated a marked increase in intracellular ROS levels after mahanimbine exposure. At 45.6 μ M, mahanimbine increased intracellular ROS levels to 182% of the control value (100%), whereas the vehicle control showed only a minor increase to 102%, which was statistically non-significant (ns). The increase induced by mahanimbine was highly statistically significant, supporting an association between mahanimbine treatment and increased intracellular ROS generation. The observed reduction in cell viability, apoptotic nuclear alterations, mitochondrial membrane potential changes, and increased intracellular ROS levels indicate that mahanimbine induces substantial cellular stress and apoptosis-associated alterations in MCF-7 breast cancer cells. These findings support the potential of mahanimbine as a promising anticancer compound; however, further mechanistic studies are required to establish the precise molecular pathways underlying

these effects and to determine its therapeutic relevance in appropriate preclinical models.

Statements & Declarations

Author Contributions

Ashapurna Sinha: Conceptualization, Investigation, Writing- original draft.

Farina Mujeeb: Supervision, Validation,

Ahamad Faiz Khan: Review, Editing,

The authors confirm that no paper mill and artificial intelligence was used.

Funding: No

Data availability: No data was used for the research described in the article.

Ethics statement: This is not required, as no human individuals were included in the study.

Competing Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Clinical trial: Not Applicable

Acknowledgment: Authors acknowledge the support extended by the Dean's Office, R&D, Integral University, Lucknow, India and providing the manuscript communication number: IU/R&D/2026-MCN0004952.

REFERENCES

1. Ahmad, A., Tiwari, R. K., Saeed, M., Ahmad, I., & Ansari, I. A. (2022). Glycyrrhizin mediates downregulation of notch pathway resulting in initiation of apoptosis and

- disruption in the cell cycle progression in cervical cancer cells. *Nutrition and Cancer*, 74(2), 622–639.
2. Alshehri, A., Ahmad, A., Tiwari, R. K., Ahmad, I., Alkhathami, A. G., Alshahrani, M. Y., Asiri, M. A., Almeleebia, T. M., Saeed, M., Yadav, D. K., & Ansari, I. A. (2022). In Vitro Evaluation of Antioxidant, Anticancer, and Anti-Inflammatory Activities of Ethanolic Leaf Extract of *Adenium obesum*. *Frontiers in Pharmacology*, Volume 13. <https://www.frontiersin.org/journals/pharmacology/articles/10.3389/fphar.2022.847534>
 3. Aniogo, E. C., George, B. P., & Abrahamse, H. (2023). Photobiomodulation improves anti-tumor efficacy of photodynamic therapy against resistant MCF-7 cancer cells. *Biomedicines*, 11(6), 1547.
 4. Asma, S. T., Acaroz, U., Imre, K., Morar, A., Shah, S. R. A., Hussain, S. Z., Arslan-Acaroz, D., Demirbas, H., Hajrulai-Musliu, Z., & Istanbulgul, F. R. (2022). Natural products/bioactive compounds as a source of anticancer drugs. *Cancers*, 14(24), 6203.
 5. Balakrishnan, R., Vijayrāja, D., Jo, S. H., Ganesan, P., Su-kim, I., & Choi, D. K. (2020). Medicinal profile, phytochemistry, and pharmacological activities of *murraya koenigii* and its primary bioactive compounds. *Antioxidants*, 9(2). <https://doi.org/10.3390/antiox9020101>
 6. Bose, D., Shetty, S., Udupi, A., & Srinivasan, K. (2025). *Murraya koenigii* as a Phytotherapeutic Agent in Breast Cancer: A Comprehensive Review. *Pharmacological Research-Natural Products*, 100487.
 7. Dehelean, C. A., Marcovici, I., Soica, C., Mioc, M., Coricovac, D., Iurciuc, S., Cretu, O. M., & Pinzaru, I. (2021). Plant-derived anticancer compounds as new perspectives in drug discovery and alternative therapy. *Molecules*, 26(4), 1109.
 8. Betts, Z., Deveci Ozkan, A., Yuksel, B., Alimudin, J., Aydin, D., Aksoy, O., & Yanar, S. (2023). Investigation of the combined cytotoxicity induced by sodium butyrate and a flavonoid quercetin treatment on MCF-7 breast cancer cells. *Journal of Toxicology and Environmental Health, Part A*, 86(22), 833–845.
 9. Hasan, A., Khamjan, N., Lohani, M., & Mir, S. S. (2023). Targeted inhibition of Hsp90 in combination with metformin modulates programmed cell death pathways in A549 lung cancer cells. *Applied Biochemistry and Biotechnology*, 195(12), 7338–7378.
 10. Hobani, Y. H. (2022). Cytotoxicity of Mahanimbine from Curry Leaves in Human Breast Cancer Cells (MCF-7) via Mitochondrial Apoptosis and Anti-Angiogenesis. *Molecules*, 27(3), 971. <https://doi.org/10.3390/molecules27030971>
 11. Hu, G., Pu, J., Wang, Q., & Zou, X. (2023). Mahanimbine Suppresses the Proliferation of Lung Cancer A549 Cells Via Inducing Intrinsic Apoptotic Pathway. *Revista Brasileira de Farmacognosia*, 33(2), 384–393. <https://doi.org/10.1007/s43450-023-00357-y>
 12. Hussien, K., Sweilam, S. H., El-Shiekh, R. A., Sayed, G. A., Hafeez, M. S. A. El, Abbas, H. A., Attallah, M. G., & Ali-Tammam, M. (2025). Carbazole Alkaloids as Antimicrobial and Antibiofilm Agents: A Focus on Plant-Derived Natural Alkaloids. *Chemistry & Biodiversity*, e00242.
 13. Jomova, K., Raptova, R., Alomar, S. Y., Alwasel, S. H., Nepovimova, E., Kuca, K., & Valko, M. (2023). Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. *Archives of Toxicology*, 97(10), 2499–2574.
 14. Manikyam, H. K., Joshi, S. K., Vakadi, S., & Patil, S. B. (2021). Anticancer activity of terpenoid saponin extract of *Psidium guajava* on MCF-7 cancer cell line using DAPI and MTT assays. *African Journal of Pharmacy and Pharmacology*, 15(12), 206–211.
 15. Marsico, M., Santarsiero, A., Pappalardo, I., Convertini, P., Chiummiento, L., Sardone, A., Di Noia, M. A., Infantino, V., & Todisco, S. (2021). Mitochondria-mediated apoptosis of HCC cells triggered by knockdown of glutamate dehydrogenase 1: perspective for its inhibition through quercetin and permethylated anigopreissin A. *Biomedicines*, 9(11), 1664.
 16. Mishra, P., Ali Ahmad, M. F., Al-Keridis, L. A., Saeed, M., Alshammari, N., Alabdallah, N. M., Tiwari, R. K., Ahmad, A., Verma, M., & Fatima, S. (2023). Methotrexate-conjugated zinc oxide nanoparticles exert a substantially improved cytotoxic effect on lung cancer cells by inducing apoptosis. *Frontiers in Pharmacology*, 14, 1194578.
 17. Moradipour, A., Dariushnejad, H., Ahmadizadeh, C., & Lashgarian, H. E. (2022). Dietary flavonoid carvacrol triggers the apoptosis of human breast cancer MCF-7 cells

- via the p53/Bax/Bcl-2 axis. *Medical Oncology*, 40(1), 46.
18. Naeem, A., Hu, P., Yang, M., Zhang, J., Liu, Y., Zhu, W., & Zheng, Q. (2022). Natural products as anticancer agents: current status and future perspectives. *Molecules*, 27(23), 8367.
19. Rego, A., Furtado, J., Silva, J. A., Alvito, P., & Motta, C. (2026). From Protocol to Analysis: Performing and Interpreting the MTT Assay. In *Food Contaminants Toxicity* (pp. 103–112). Springer.
20. Samanta, S. K., Choudhury, P., Kandimalla, R., Aqil, F., Moholkar, D. N., Gupta, R. C., Das, M., Gogoi, B., Gogoi, N., & Sarma, P. P. (2024). Mahanine mediated therapeutic inhibition of estrogen receptor- α and CDK4/6 expression, decipher the chemoprevention-signaling cascade in preclinical model of breast cancer. *Journal of Ethnopharmacology*, 319, 117235.
21. Sibuh, B. Z., Khanna, S., Taneja, P., Sarkar, P., & Taneja, N. K. (2021). Molecular docking, synthesis and anticancer activity of thiosemicarbazone derivatives against MCF-7 human breast cancer cell line. *Life Sciences*, 273, 119305.
22. Smolarz, B., Nowak, A. Z., & Romanowicz, H. (2022). Breast cancer—epidemiology, classification, pathogenesis and treatment (review of literature). *Cancers*, 14(10), 2569.
23. Tsang, J. Y. S., & Gary, M. T. (2020). Molecular classification of breast cancer. *Advances in Anatomic Pathology*, 27(1), 27–35.
24. Ullah, I., Khalil, A. T., Ali, M., Iqbal, J., Ali, W., Alarifi, S., & Shinwari, Z. K. (2020). Green-synthesized silver nanoparticles induced apoptotic cell death in MCF-7 breast cancer cells by generating reactive oxygen species and activating caspase 3 and 9 enzyme activities. *Oxidative Medicine and Cellular Longevity*, 2020(1), 1215395.
25. Venugopal, J., Panneerselvam, S., & Theivendren, P. (2026). Breast Cancer Insights: Significance of *Murraya koenigii* and Their Potential in Prevention and Therapeutic Intervention. *PharmaNutrition*, 100478.
26. Wang, J., & Wu, S.-G. (2023). Breast cancer: an overview of current therapeutic strategies, challenge, and perspectives. *Breast Cancer: Targets and Therapy*, 721–730.