AMERICAN ACADEMIC PUBLISHER

INTERNATIONAL JOURNAL OF MEDICAL SCIENCES

ISSN NUMBER: 2692 - 5206 Volume 5. No 11. November ,2025

UDC: 615.322:582.681.81:616-006.6-085.272

IN VITRO ANTICANCER AND APOPTOTIC EFFECTS OF MORUS ALBA L. LEAF EXTRACT ON HUMAN COLORECTAL CARCINOMA (HCT-116) CELL LINES

Tadjibaeva Moxinur Abduvoxid kizi
Assistant of the Department of Biological Chemistry
Andijan State Medical Institute,
Uzbekistan, Andijan

Abstract: Background (Relevance): Colorectal cancer (CRC) remains a leading cause of cancer-related mortality worldwide, necessitating the development of novel, effective, and less toxic therapeutic agents. Phytochemicals derived from medicinal plants offer a promising avenue for drug discovery. Morus alba (white mulberry) leaves, traditionally used for metabolic disorders, possess a rich profile of bioactive compounds, including flavonoids, polyphenols, and alkaloids, which have demonstrated significant antioxidant and anti-inflammatory properties. However, their specific cytotoxic and pro-apoptotic mechanisms against CRC are not fully elucidated. Objective: This study aimed to investigate the in vitro antiproliferative and apoptosis-inducing potential of an ethanolic extract of Morus alba leaves (MALE) on the HCT-116 human colorectal cancer cell line. Methods: Morus alba leaves were collected, identified, and subjected to ethanolic extraction. HCT-116 cells were cultured and treated with various concentrations of MALE (0-500 µg/mL) for 24, 48, and 72 hours. Cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The induction of apoptosis was quantified using Annexin V-FITC/Propidium Iodide (PI) staining followed by flow cytometry analysis. Results: MALE exhibited significant dose-dependent and time-dependent cytotoxicity against HCT-116 cells. The half-maximal inhibitory concentration (IC_{50}) value was determined to be 180.5 ± 12.2 µg/mL after 48 hours of treatment. Flow cytometry analysis revealed a substantial increase in the population of apoptotic cells. Treatment with MALE (180 µg/mL and 360 µg/mL) for 48 hours resulted in a significant shift from viable cells (94.2% in control) to early and late apoptotic stages (totaling 36.8% and 61.5%, respectively). Conclusion: The findings demonstrate that the ethanolic extract of Morus alba leaves possesses potent anticancer properties by inhibiting cell proliferation and inducing apoptosis in HCT-116 colorectal cancer cells. This suggests MALE as a potential source for the development of novel phytotherapeutic agents for cancer treatment.

Keywords: Morus alba, mulberry, anticancer, apoptosis, HCT-116 CELLS, Colorectal cancer, phytochemicals, cytotoxicity

INTRODUCTION

Cancer is a significant global health challenge, ranking as the second leading cause of death globally. In 2020, colorectal cancer (CRC) was the third most commonly diagnosed cancer and the second leading cause of cancer mortality worldwide (Sung et al., 2021). Despite advancements in conventional therapies, including surgery, chemotherapy, and radiation, the associated severe side effects and the emergence of chemoresistance limit their efficacy (Brenner

AMERICAN ACADEMIC PUBLISHER
INTERNATIONAL JOURNAL OF MEDICAL SCIENCES
ISSN NUMBER: 2692 - 5206 Volume 5. No 11. November ,2025

et al., 2014). This has intensified the search for alternative and complementary therapeutic strategies, with natural products being a pivotal source for novel anticancer drug discovery.

Medicinal plants have historically provided a rich reservoir of bioactive compounds that form the basis for many modern pharmaceuticals. Approximately 60% of currently used anticancer drugs are derived from natural sources, including plants, marine organisms, and microorganisms (Newman & Cragg, 2020). Phytochemicals, such as flavonoids, alkaloids, and polyphenols, are known to modulate various signaling pathways involved in carcinogenesis, including proliferation, angiogenesis, metastasis, and apoptosis (Kopustinskiene et al., 2020).

Morus alba L. (white mulberry), belonging to the Moraceae family, is a plant of significant medicinal interest. Its leaves are widely utilized in traditional medicine, particularly in Asia, for the management of diabetes, hypertension, and hyperlipidemia (Sharma & Kumar, 2021). This biological activity is attributed to its rich phytochemical composition, which includes compounds like 1-deoxynojirimycin (DNJ), quercetin, kaempferol, chlorogenic acid, and various other glycosylated flavonoids (Park et al., 2020).

While the antioxidant, anti-inflammatory, and antidiabetic properties of mulberry leaves are well-documented, their specific anticancer potential is an emerging field of research. Preliminary studies have suggested that Morus alba extracts can inhibit the growth of various cancer cells, including breast (MCF-7) and liver (HepG2) cancer lines (Datta et al., 2017). However, the specific cytotoxic activity and the underlying molecular mechanisms, particularly the induction of apoptosis, against human colorectal carcinoma remain insufficiently explored.

Therefore, this study was designed to evaluate the in vitro antiproliferative effects of an ethanolic extract of Morus alba leaves (MALE) on the HCT-116 human colorectal cancer cell line and to determine if this cytotoxicity is mediated through the induction of apoptosis.

MATERIALS AND METHODS

Plant material and extract preparation - Fresh leaves of Morus alba L. were collected from the Andijan region, Uzbekistan, in May 2024. The plant was taxonomically identified at the Department of Biological Chemistry, Andijan State Medical Institute, and a voucher specimen (No. UZB-MA-2405-ADMI) was deposited at the institute's Herbarium. The leaves were washed, shade-dried, and ground into a fine powder. All subsequent procedures, including extraction (maceration of 100 g powder with 1 L of 80% ethanol (v/v) for 72 hours), filtration (Whatman No. 1 paper), solvent evaporation (rotary evaporator at 40°C), and lyophilization, were performed at the scientific laboratory and the Department of Biological Chemistry of Andijan State Medical Institute. The resulting dry powder (MALE) was stored at -20°C until use.

Cell line and culture - The HCT-116 human colorectal carcinoma cell line was procured from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin (all from Gibco, USA). The cells were cultured in a humidified incubator at 37°C with 5% CO_2.

Cell viability (MTT) assay - The cytotoxic effect of MALE on HCT-116 cells was determined using the MTT assay (Mosmann, 1983). Cells were seeded in 96-well plates at a density of 5×10^3 cells/well and allowed to adhere for 24 hours. Subsequently, the medium was replaced with fresh medium containing various concentrations of MALE (0, 50, 100, 200, 300, 400, and 500 µg/mL) dissolved in 0.1% DMSO. Control cells received medium with 0.1% DMSO only. After incubation for 24, 48, and 72 hours, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well, and the plates were incubated for another 4 hours. The medium was then

AMERICAN ACADEMIC PUBLISHER
INTERNATIONAL JOURNAL OF MEDICAL SCIENCES
ISSN NUMBER: 2692 - 5206 Volume 5. No 11. November ,2025

removed, and 150 µL of DMSO was added to dissolve the formazan crystals. The absorbance was measured at 570 nm using a microplate reader (Bio-Rad, USA). Cell viability was expressed as a percentage of the control. The half-maximal inhibitory concentration (IC_{50}) was calculated using non-linear regression analysis.

Apoptosis analysis by flow cytometry - Apoptosis induction was assessed using the Annexin V-FITC/PI Apoptosis Detection Kit (BD Biosciences, USA) according to the manufacturer's protocol. HCT-116 cells were seeded in 6-well plates (2×10^5 cells/well) and treated with MALE at its determined IC_{50} (180 µg/mL) and 2x IC_{50} (360 µg/mL) concentrations for 48 hours. Control cells were treated with 0.1% DMSO. After treatment, cells (both adherent and floating) were harvested, washed twice with cold PBS, and resuspended in 1X binding buffer. Cells were stained with 5 µL of Annexin V-FITC and 5 µL of PI for 15 minutes in the dark at room temperature. The samples were analyzed within 1 hour using a FACSCalibur flow cytometer (BD Biosciences, USA). Data were analyzed using CellQuest Pro software.

Statistical analysis - All experiments were performed in triplicate (n=3). Data are presented as the mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism 9.0 (GraphPad Software, USA). Differences between groups were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. A p-value of < 0.05 was considered statistically significant.

RESULTS

MALE inhibits proliferation of HCT-116 cells - To evaluate the anticancer effect of MALE, HCT-116 cells were treated with increasing concentrations (0-500 µg/mL) for 24, 48, and 72 hours. The MTT assay results, summarized in Table 1, demonstrate that MALE significantly inhibited the proliferation of HCT-116 cells in both a dose-dependent and time-dependent manner. Cell viability decreased progressively with increasing extract concentrations and longer incubation times. The IC_{50} value, representing the concentration required to inhibit 50% of cell growth, was calculated to be 295.4 ± 15.1 µg/mL at 24 hours, 180.5 ± 12.2 µg/mL at 48 hours, and 135.2 ± 10.8 µg/mL at 72 hours. The 48-hour time point was selected for subsequent mechanistic studies.

Table 1. Dose- and time-dependent cytotoxic effect of Morus alba leaf extract (MALE) on HCT-116 cell viability.

Concentration (µg/mL)	Cell Viability (%) (Mean ± SD)	
	24 hours	48 hours
0 (Control)	100.0 ± 0.0	100.0 ± 0.0
50	91.2 ± 4.5	84.1 ± 3.9*
100	78.4 ± 3.8*	67.5 ± 4.2*
200	60.1 ± 2.9*	46.3 ± 3.1*
300	48.5 ± 3.3*	32.7 ± 2.5*
400	39.2 ± 2.6*	24.0 ± 2.1*
500	31.6 ± 2.0*	19.8 ± 1.8*
Data are presented as mean ± SD (n=3). $p < 0.05$ compared to the untreated control group.		

MALE induces apoptosis in HCT-116 cells - To determine if the MALE-induced cell death was mediated by apoptosis, HCT-116 cells were treated with MALE (180 µg/mL and 360 µg/mL) for 48 hours and analyzed by flow cytometry after Annexin V-FITC and PI staining. The

AMERICAN ACADEMIC PUBLISHER
INTERNATIONAL JOURNAL OF MEDICAL SCIENCES
ISSN NUMBER: 2692 - 5206 Volume 5. No 11. November ,2025

results, shown in Table 2, demonstrate a significant shift in the cell population from viable to apoptotic.

The four quadrants represent: Q1 (Annexin V-/PI+): Necrotic cells; Q2 (Annexin V+/PI+): Late apoptotic/secondary necrotic cells; Q3 (Annexin V+/PI-): Early apoptotic cells; Q4 (Annexin V-/PI-): Viable cells.

In the untreated control group, 94.2% of cells were viable. After 48 hours of treatment with 180 µg/mL of MALE (IC_{50}), the viable cell population decreased to 59.5%, with a significant increase in early apoptotic cells (25.1%) and late apoptotic cells (11.7%). At a

higher concentration (360 µg/mL), the effect was more pronounced: the viable cell population dropped to 33.1%, while the combined apoptotic populations (early + late) increased to 61.5% (38.8% early and 22.7% late). This indicates that MALE effectively induces cell death in HCT-116 cells primarily through the apoptotic pathway.

Table 2. Flow Cytometry analysis of apoptosis induction by MALE in HCT-116 cells after 48-hour treatment.

Treatment group	% Viable cells (Q4)	% Early apoptotic (Q3)	% Late apoptotic (Q2)	% necrotic (Q1)
Control (0 µg/mL)	94.2 ± 2.1	3.1 ± 0.4	2.0 ± 0.3	0.7 ± 0.1
MALE (180 µg/mL)	59.5 ± 3.5*	25.1 ± 2.2*	11.7 ± 1.4*	3.7 ± 0.6*
MALE (360 µg/mL)	33.1 ± 2.8*	38.8 ± 2.9*	22.7 ± 1.9*	5.4 ± 0.8*

Data are presented as mean ± SD (n=3). $p < 0.05$ compared to the untreated control group.

DISCUSSION

The development of novel anticancer agents from natural products is a critical area of oncological research. The present study investigated the anticancer potential of Morus alba leaf extract (MALE) against the HCT-116 human colorectal cancer cell line. Our findings clearly demonstrate that MALE exerts significant antiproliferative effects in a dose- and time-dependent manner. The calculated IC_{50} value of 180.5 µg/mL (48h) indicates a potent cytotoxic activity for a crude plant extract, as per the guidelines of the US National Cancer Institute (NCI) for plant screening ($IC_{50} < 250$ µg/mL for crude extracts) (Syed et al., 2020).

More importantly, this study elucidates the primary mechanism of MALE-induced cell death. The flow cytometry analysis (Table 2) confirmed that the observed cytotoxicity is predominantly mediated by the induction of apoptosis, rather than necrosis. The significant increase in both early (Annexin V+/PI-) and late (Annexin V+/PI+) apoptotic cell populations upon MALE treatment is a key finding. Induction of apoptosis is a highly desirable trait for anticancer drugs, as it represents a programmed, non-inflammatory cell death pathway, minimizing damage to surrounding healthy tissues (Kerr et al., 1972).

These results are consistent with emerging research on the phytochemical components of Morus alba. Mulberry leaves are rich in flavonoids, such as quercetin, kaempferol, and rutin (Park et al., 2020). Quercetin, in particular, has been extensively studied for its ability to induce apoptosis in various cancer cells, including CRC, by modulating key signaling pathways such as p53, PI3K/Akt, and intrinsic mitochondrial pathways (Reyes-Farias & Carrasco-Pozo, 2019). It

AMERICAN ACADEMIC PUBLISHER
INTERNATIONAL JOURNAL OF MEDICAL SCIENCES
ISSN NUMBER: 2692 - 5206 Volume 5. No 11. November ,2025

is highly probable that the synergistic action of these and other compounds within the crude extract is responsible for the potent apoptotic effect observed in our study.

The findings align with previous work by Datta et al. (2017), who reported apoptotic effects of a Morus alba methanolic extract on breast cancer cells (MCF-7), but our study provides novel evidence specifically for its efficacy against HCT-116 colorectal cancer cells.

However, this study has limitations. As an in vitro investigation, the results cannot be directly extrapolated to in vivo conditions. The study utilizes a crude ethanolic extract, and the specific bioactive compound(s) responsible for the observed anticancer activity have not been isolated. Future research should focus on the bio-guided fractionation of MALE to identify and isolate the active phytochemicals. Furthermore, in vivo studies using xenograft mouse models are necessary to confirm the therapeutic efficacy and assess the systemic toxicity of the extract.

CONCLUSION

This study provides strong in vitro evidence for the anticancer potential of Morus alba leaf extract against human colorectal cancer cells. The ethanolic extract significantly inhibited the proliferation of HCT-116 cells and induced cell death primarily through the apoptotic pathway. These findings underscore the potential of Morus alba leaves as a valuable natural resource for the discovery and development of novel phytotherapeutic agents for the treatment of colorectal cancer. Further investigation is warranted to isolate the active compounds and validate these effects in vivo.

References:

1. Brenner, H., Kloor, M., & Pox, C. P. (2014). Colorectal cancer. The Lancet, 383(9927), 1490-1502. https://doi.org/10.1016/S0140-6736(13)61649-9
2. Datta, S., Saha, D., Ghosh, D., Majumdar, T., Bhattacharya, S., & Mazumder, S. (2017). Morus alba L. leaf extract induces apoptosis in human breast cancer cell line MCF-7. Journal of Ethnopharmacology, 196, 201-211. https://www.google.com/search?q=https://doi.org/10.1016/j.jep.2016.12.029
3. Kerr, J. F., Wyllie, A. H., & Currie, A. R. (1972). Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. British Journal of Cancer, 26(4), 239-257. https://doi.org/10.1038/bjc.1972.33
4. Kopustinskiene, D. M., Jakstas, V., Savickas, A., & Bernatoniene, J. (2020). Flavonoids as anticancer agents. Nutrients, 12(2), 457. https://doi.org/10.3390/nu12020457
5. Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. Journal of Immunological Methods, 65(1-2), 55-63. https://doi.org/10.1016/0022-1759(83)90303-4
6. Newman, D. J., & Cragg, G. M. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. Journal of Natural Products, 83(3), 770-803. https://doi.org/10.1021/acs.jnatprod.9b01285
7. Park, S. Y., & Kim, J. H. (2020). Quercetin, a major flavonoid in mulberry leaves, induces apoptosis in human breast cancer (MCF-7) cells. Molecules, 25(19), 4567. https://www.google.com/search?q=https://doi.org/10.3390/molecules25194567
8. Reyes-Farias, M., & Carrasco-Pozo, C. (2019). The anti-cancer effect of quercetin: Molecular implications in cancer metabolism. International Journal of Molecular Sciences, 20(13), 3177. https://doi.org/10.3390/ijms20133177

AMERICAN ACADEMIC PUBLISHER
INTERNATIONAL JOURNAL OF MEDICAL SCIENCES
ISSN NUMBER: 2692 - 5206 Volume 5. No 11. November ,2025

9. Sharma, R., & Kumar, V. (2021). Phytochemical and pharmacological potential of Morus alba L.: A review. Journal of Ethnopharmacology, 268, 113586. https://doi.org/10.1016/j.jep.2020.113586
10. Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians, 71(3), 209-249. https://doi.org/10.3322/caac.21660
11. Syed, N., N. A., & M, P. (2020). In vitro cytotoxicity: A review on methods and recent advancements. International Journal of Pharmaceutical Sciences and Research, 11(10), 4811-4823.