ORIGINAL ARTICLE

Evaluation of Anti-Cancer Activity of *Momordica dioica* using MTT and DAPI Assays

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Abstract

Aim: The aim of this study was to evaluate the anti-cancer potential of methanolic extracts from the stem-leaf, root, and callus of *Momordica dioica* against two cancer cell lines: Michigan Cancer Foundation-7 (MCF-7) (human breast cancer) and A549 (lung cancer). **Materials and Methods:** The evaluation was conducted using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and 4′,6-Diamidino-2-phenylindole (DAPI) staining method, which are reliable and widely used techniques in cancer research. The MTT assay, a sensitive and quantitative colorimetric method, assessed cell viability, proliferation, and metabolic activity. **Results and Discussion:** The results revealed that methanolic stem-leaf and callus extracts significantly inhibited the growth of MCF-7 cells at IC_{50} concentrations, indicating their effectiveness in targeting breast cancer cells. DAPI staining, a fluorescence-based method used to detect DNA fragmentation and apoptosis, was applied to the A549 lung cancer cell line. The findings demonstrated a dose-dependent induction of apoptosis in A549 cells treated with the extracts, suggesting their potential role in activating apoptotic pathways in lung cancer cells. Overall, these results highlight the promising anti-cancer properties of *M. dioica* methanolic extracts. **Conclusion:** The plant extract can inhibit cancer cell growth and induce apoptosis, underscoring their potential as natural therapeutic agents. This study provides a foundation for further research aimed at isolating and characterizing the active compounds responsible for these effects, which could lead to the development of novel and effective anti-cancer drugs.

Key words: A549, anti-cancer, 4',6-diamidino-2-phenylindole, Michigan cancer foundation-7, *Momordica dioica*, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

INTRODUCTION

Cancer is a collection of diseases that cause abnormal cells to develop and spread uncontrollably. It is an important cause of death for people globally. Cancer has been prevented using natural products derived from fruits and vegetables.[1] Plant-derived chemicals have long been a valuable source of medications for a range of illnesses, and in recent times, their many pharmacological qualities such as their cytotoxic and cancer-chemopreventive effects have drawn a lot of attention Bertuccio.[2] An estimated 50% or more of anticancer medicines are thought to be plant-derived chemicals in one form or another.[3] In the *Cucurbitaceae* family, *Momordica dioica* is a perennial climber that is dioecious. In India, it is known by several names such as Kankro, Kartoli, Kantola, Kantroli, ban Karola, or Janglee Karela. In general, it is called spiny gourd, teasel gourd, or little bitter gourd worldwide. Conventionally, it is being used for pick up the check eye illnesses, poisoning, and fever. Fruits, leaves, and tuberous roots are used as a popular remedy for diabetes.[4,5] The plant was reported to have anti-diabetic, analgesic, postcoital anti-fertility, nematocidal, anti-allergic, analgesic, anti-malarial, anti-feedant, anti-bacterial, anti-oxidants and hepatoprotective, jaundice, and bleeding pile possessions.[6] In nature, male and female plants are shown collected at a ratio of 1:15. Due to the above-declared reason, the natural population of the plant is regularly decreasing, and hence, conservation of this plant is compulsory. In this way, plant biotechnology

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plays an alternative tool for large scale duplication. It is clear from the literature review that no research has been done to assess the anti-cancer potential of either the callus or the stem-leaf extract[7] The anticancer action of the active components in the dichloromethane extract of *M. dioica* roots (L1210) has been demonstrated by pharmacological tests on cancer cells. It was demonstrated that at a dosage of 4 µg/mL, the growth inhibitory index (%) was 50%.[8] Therefore, after treating A549 cancer cells with the extract, the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test was used to measure the inhibition of cell growth in Michigan Cancer Foundation-7 (MCF-7), and the 4′,6-Diamidino-2-phenylindole (DAPI) technique was used to assess the anti-cancer activity of the callus and stem-leaf methanolic extract of *M. dioica*.[9] The MTT assay based on the potential of mitochondrial dehydrogenase enzymes in living cells to change MTT, a yellow water-soluble substrate, into a dark blue formazan product that is water insoluble.[10] When the tetrazolium ring breaks, living cells can change the yellow MTT into a water-insoluble purple-blue compound that precipitates in the cytoplasm of the cells and can be dissolved after cell lysis. However, dead cells that have suffered toxic damage are unable to change MTT, and the DAPI method measures the proportion of dying cells compared to control cells that were not treated. The present study focuses on preparing extract and after successful callus formation; its extract was tested for its anti-cancerous activity on two cell lines, that is, A549 and MCF-7 using MTT and DAPI assay methods. The results of this experiment could provide insight into the potential use of these extracts for various applications, such as developing new drugs or supplements with apoptotic effects.

MATERIALS AND METHODS

The *M. dioica* fruits were gathered from the Vindhya region in Satna (MP) and verified by the Deendayal Research Institute in Chitrakoot (MP).

Preparation of extract

The collected plant was cleaned with distilled water, sun-dried, ground into a coarse powder, and then extracted with different solvents using the Soxhlet extraction technique. This procedure involved taking 20 g of powder from each explant, packing it neatly into a thimble, and then extracting the material using 250 mL of various solvents individually. Gases such as petroleum ether, benzene, methanol, and chloroform were employed. The entire experimentation continues for up to 24 h or until the appearance of the color solvent. The extracted liquid was collected in a beaker and heated at 30–40°C until all the solvent evaporated. The collected tincture was stored at 4°C for future study.

Cell culture

Cultured cancer cells are essential reagents for both quickly identifying possible anti-cancer drugs and elucidating how they work. The cell lines utilized in this investigation were human breast cancer (MCF-7) and lung cancer (A549).

Anti-cancer activity on cancer cell lines

The anticancer activity of *M. dioica* extract was evaluated using two assays: The MTT assay on MCF-7 cell lines to assess cell viability and the DAPI staining assay on A549 cell lines to examine nuclear morphology and apoptosis. These methods provide insights into the extract's cytotoxic and apoptotic effects.

MTT assay

M. dioica root, stem-leaf, and callus extracts were investigated for their anti-cancerous properties using the MCF-7 cell line. This cell line was cultivated in Dulbecco's modified Eagle medium, which was enhanced with 10% fetal bovine serum (10%, GIBCO), antibiotic Penicillin and Streptomycin solution, and incubated at 37°C in a 5% CO_2 humidified environment. The cells were seeded in 96-well microtiter plates with a total volume of 100 µL. The monolayer of cells in the plate was exposed to various concentrations of the chloroform and methanolic root, stem-leaf, and callus extracts running from 0.01, 0.025, and 0.1 µL/mL. The cells were incubated for 24 h. The medium was detached and the cells were washed with phosphate-buffered saline (PBS) (pH 7.4). MTT assay was performed to determine the cell viability which was measured by the reduction of MTT to a purple-colored formazan product. Using the formulas, the concentration needed to provide a 50% suppression of cell viability (IC_{50}) was calculated.

The concentration of compound required to inhibit 50% cell growth, which was determined by plotting a log graph (concentration of the compound) versus % cell inhibition. Percentage inhibition of the test compounds against all cell lines was calculated using the formula:

$$\% \text{ cell survival} = \frac{(At - Ab)}{(Ac - Ab)} \times 100 \quad \text{(i)}$$

$$\% \text{ cell inhibition} = 100 - \% \text{ cell survival} \quad \text{(ii)}$$

Where, A_t = Absorbance of test, A_b= Absorbance of Blank (Media), A_c= Absorbance of control (cells)

The MTT assay used the viability and proliferation of the test cells to detect the inhibitory effect of the test substance. It was shown that when the test compound's concentration increased, the percentage of cells that survived decreased. After the MTT assay of all extracts, we applied the DAPI method for

better results of extract, one is stem-leaf methanolic extract and another is tissue cultured callus methanolic extract.

DAPI assay

The DAPI method for detecting apoptosis in A549 lung cancer cells[11] involves the use of Annexin V-FITC and propidium iodide (PI) staining, as commonly employed in flow cytometry.[12] The process begins with cell culture and treatment, where A549 cells are cultured in an appropriate medium under standard conditions (37°C, 5% CO_2). Two sets of cultures are prepared: One as an untreated control and the other treated with extracts at concentrations of 5, 10, and 15 µg/mL. The cultures are incubated to allow the extracts to exert their effects.

Next, cells are harvested by removing the culture medium, rinsing twice with PBS, and detaching the cells using trypsin. The detached cells are collected through centrifugation at 200 × g for 5 min, and the supernatant is discarded. Following this, staining is performed by resuspending the cell pellet in a binding buffer and adjusting the cell concentration to 1×10^6 cells/mL. Annexin V-FITC and PI are added, and the cells are incubated in the dark for 15 min at room temperature.

For flow cytometry analysis, 400 µL of binding buffer is added to each sample, and the stained cells are analyzed. Annexin V-FITC is excited using a 488 nm argon laser and detected in the FL1 channel (530/30 nm), while PI is excited with a 561 nm red laser and detected in the FL2 channel (585/42 nm). Gating strategies are applied to distinguish live, apoptotic, and necrotic cells. Finally, the data are analyzed using appropriate software (e.g., FlowJo) to calculate the proportions of Annexin V-positive/PI-negative cells (early apoptotic), Annexin V-positive/PI-positive cells (late apoptotic/necrotic), and Annexin V-negative/PI-negative cells (live). Results are visualized as histograms or scatter plots to compare untreated controls with treatment groups at different extract concentrations.

RESULTS AND DISCUSSION

The millions of widely accessible plant-based chemicals have enabled the development of several clinically effective anti-cancer drugs. When examining the anti-cancer properties of the crude root, stem-leaf and callus extract, and chloroform root extract, the primary goals are to either isolate bioactive agents that can be used directly as anti-cancer medications or identify bioactive compounds that can be utilized as lead substances in the development of semi-synthetic anti-cancer medications. In the present investigation, *M. dioica* plant stem-leaf, root, and callus extracts were prepared using methanol and chloroform as solvents. It is well documented that methanol is commonly used as a solvent for plant extract preparation for evaluating the anti-cancer activity in several plant species; in this study,

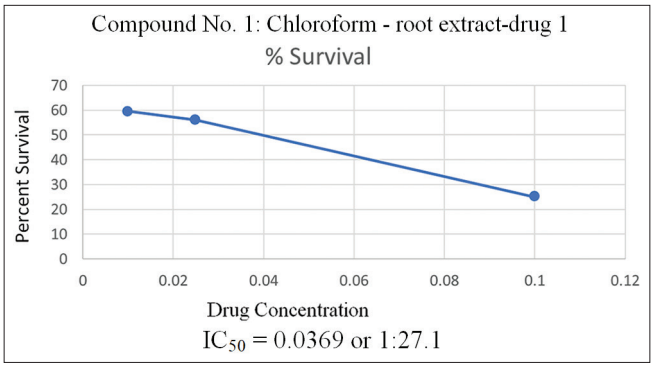

Figure 1: Chloroform root extract of *Momordica dioica*

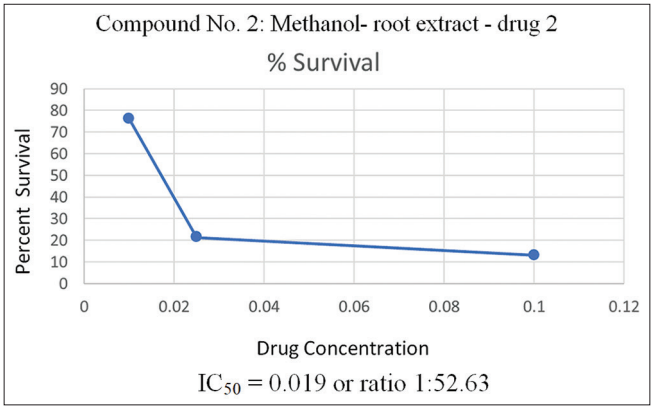

Figure 2: Methanol root extract of *Momordica dioica*

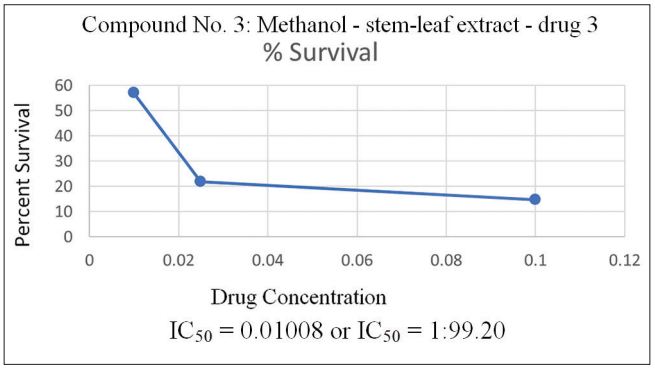

Figure 3: Methanol stem-leaf of *Momordica dioica*

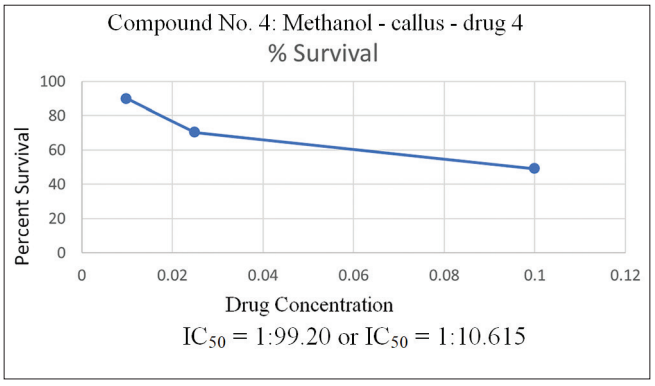

Figure 4: Methanol callus extract of *Momordica dioica*

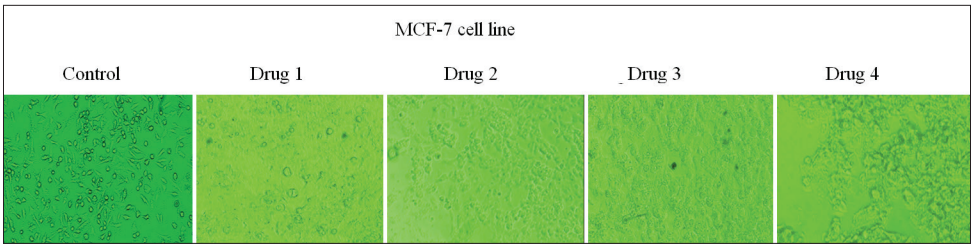

Figure 5: Photomicrographs of drug samples with control using cancer cell lines

we express the anti-cancer perspective of the methanolic stem-leaf, root, and callus extract and chloroform root extract of *M. dioica* in well categorized MCF-7 and A549 cell lines.

MTT (MCF-7)

The anti-proliferative effects of *M. dioica* methanolic stem-leaf, root, and callus extract as well as chloroform root extract on the growth of human breast cancer cell line were examined using the MTT assay, which demonstrated higher inhibition toward the tested carcinoma cell line[1] which observed that the MTT assay was conducted to investigate the anticancer effect of the methanolic extract of *M. dioica* against human carcinoma cell lines at a concentration of 100 µL/mL. In the experiment, we used three concentrations of extracts that are 0.01 µL/mL, 0.025 µL/mL, and 0.1 µL/mL and we got increasing inhibition results against cancerous cells on 0.1 µL/mL concentration. In the result of extracts, chloroform root extract Drug-1 showed anti-cancer activity by causing 75% inhibition of MCF-7 cells at IC_{50} value of 0.1 µL/mL concentration that clearly shows its superiority as in research where *Nepeta deflersiana* extract was used on the same line but they have achieved 99% inhibition at 100 ug/mL concentration.[13] and methanolic root extract Drug-2 showed anti-cancer activity by causing 87% inhibition at IC_{50} value of 0.1 µL/mL concentration which aligns with previous research where hydroalcoholic extraction of the bitter melon where the cell viability decreases as the concentration increases from 100 to 500 ug/mL.[14] Drug-3's stem-leaf extract in methanol demonstrated exceptional anti-cancer properties through its 86% inhibition rate using an IC_{50} value of 0.1 µL/mL concentration thus exceeding previous findings of Pseudocedrelakotschyi methanol extract testing on MCF-7 cell lines which exhibited decreasing cell viability from 80 to 90 µL/mL.[15] The anticancer effects of Drug-3 reach a significant level when used at concentrations that are substantially lower than those required for Pseudocedrelakotschyi extract. Last methanolic tissue cultured callus extract Drug-4 showed anticancer activity by causing 51.1% inhibition of MCF-7 cells at IC_{50} value of 0.1 µL/mL concentration which aligns with previous research where the methanol extract of *E. guineensis* exhibited significant activity against the MCF-7 cell line with an IC_{50} value of 15.00–20 µg/mL.[16] [Figures 1-5][1] observed that the MTT assay was conducted to investigate the anticancer effect of the methanolic extract of *M. dioica* against human

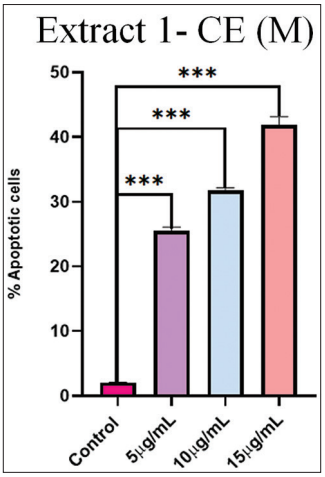

Figure 6: Callus extract- Methanol (CE-M) of *Momordica dioica*

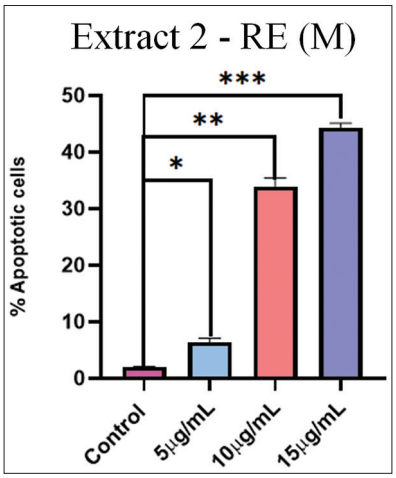

Figure 7: Root extract-methanol (RE-M) of *Momordica dioica*

carcinoma cell lines at a concentration of 100 µL/mL.[17] The MTT assay functions as a colorimetric method to evaluate cell viability according to reported research.[18] The antiproliferative activity of Ganoderma lucidum alcoholic extract showed 70% inhibition against MCF-7 cells when using 500 µg/mL concentration. The ethanol fractions from inula viscose flowers and ononishirta aerial parts showed anticancer effects against MCF-7 cells with IC_{50} values of 5.78 µg/mL and 27.96 µg/mL respectively.[19] Plants extracts show strong potential as natural anticancer agents for breast cancer treatment because they demonstrate promising therapeutic effectiveness while producing fewer side effects than standard chemotherapy.

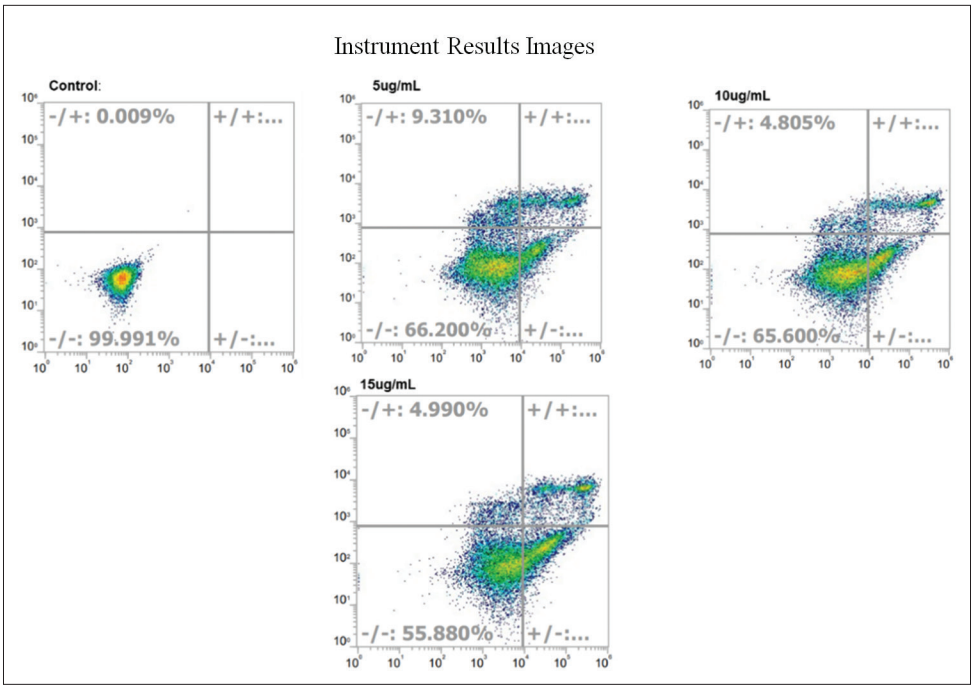

Figure 8: 4′,6-Diamidino-2-phenylindole stanning test of methanol callus extract (CE-M) using flow cytometry

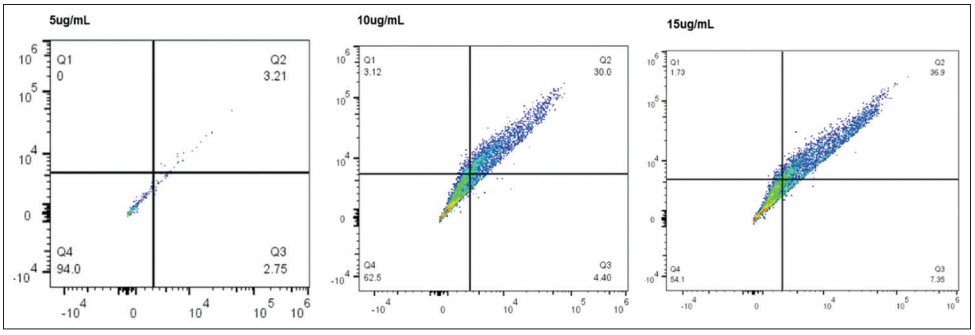

Figure 9: 4′,6-Diamidino-2-phenylindole stanning test of methanol root extract (RE-M) using flow cytometry

DAPI (A549)

DAPI staining is used to observe the nuclear contents in shrunken cells.[20,21] Research findings demonstrated that DAPI showed pamidronate effects together with antiproliferative properties and apoptotic abilities and anti-migratory characteristics against hepatocellular cancer cells. The research evaluated the apoptotic effects on A549 cell line (lung cancer cells) using Extract 1 [Figure 8] derived from tissue cultured callus methanolic extraction and Extract 2 [Figure 9] derived from *M. dioica* root methanolic extraction.[22] Apoptosis is a crucial process of programmed cell death that plays an essential role in maintaining tissue homeostasis, eliminating damaged or infected cells, and preventing the formation of cancerous cells. Flow cytometry is a widely used technique to measure apoptosis by detecting the changes in the cell membrane and intracellular markers.[23] In this study, the researchers investigate the effect of two extracts on inducing apoptosis in cells using flow cytometry. The results showed that the apoptosis induced by the extracts at 5 ug/mL, 10 ug/mL, and 15 ug/mL was higher than the control, and the effect was dose-dependent. Furthermore, the researchers analyzed the statistical significance of the results using *P*-value.[24] *P*-value is a measure of the probability of obtaining the observed result by chance. $P < 0.05$ is considered significant, indicating that the results are unlikely to have occurred by chance. In this study, Extract 1 had *P*-value of three stars, indicating a high statistical significance level [Figure 6].[25] Extract 2 had *P*-value with one star at 5 ug/mL, two stars at 10 ug/mL, and three stars at 15 ug/mL, indicating a significant dose-dependent effect [Figure 7]. The significance of stars in *P*-value is a graphical representation of the statistical significance of the results.[26] The number of stars represents the level of consequence, with three stars indicating a high level of significance, two stars indicating moderate significance, and one star indicating low significance.

CONCLUSION

The study showed that the four extracts have a significant investigation of the antiproliferative activity of *M. dioica*,

methanolic and stem-leaf, root and callus extract, and chloroform root extract on the growth of human breast carcinoma cell lines by MTT assay which showed increased inhibition toward the tested carcinoma cell line. The best two extracts were selected for the DAPI staining method, which has a significant dose-dependent effect in inducing apoptosis, as indicated by the higher level of apoptosis compared to the control and the significant *P*-value with stars. The results of this research could influence the creation of novel therapeutic drugs for the treatment of cancer.

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