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Original Article

Betanin and Resveratrol as Dual Modulators of Apoptotic Signaling in Oral (KB) and Osteosarcoma (143B) Cells

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ABSTRACT

Background: Oral Squamous Cell Carcinoma (OSCC) is characterised by its ability to evade apoptosis, contributing to therapeutic resistance and poor clinical outcomes. Natural compounds such as betanin and resveratrol have demonstrated pro-apoptotic effects in various cancer models. This study investigates the apoptotic potential of betanin and resveratrol, individually and in combination, in KB oral cancer cells through modulation of mitochondrial apoptotic pathways. **Materials and Methods:** KB cells and 143B cells were treated with betanin (10 µM and 25 µM), resveratrol (10 µM 25 µM), and their combination (10 µM each) were administered to KB cells and 143B cells. The MTT test was utilized to measure cell viability, and phase-contrast microscopy was used to look at morphological alterations. Gene expression of genes was related to apoptotic regulators such as p53, Bax, Bcl-2, caspase-9 enzyme activities were evaluated using RT-PCR techniques. **Results:** Both betanin and resveratrol markedly reduced cell effectiveness varies with the dose, with the combination treatment inducing the greatest cytotoxic effect. Morphological assessment revealed classic apoptotic features, including cell shrinkage, rounding, and detachment. Gene expression studies indicated upregulation of p53, Bax, caspa see-3 and caspase-9 activity, particularly in the co-treatment group, suggesting enhanced engagement of the intrinsic apoptosis route. **Conclusion:** Betanin and resveratrol reduced cell viability in a dose-dependent manner, with the combination exerting the strongest cytotoxicity, marked by apoptotic morphology and significant upregulation of p53, Bax, caspase-3, and caspase-9, indicating activation of the intrinsic apoptotic pathway. These results highlight the therapeutic promise of these naturally derived compounds and suggest their potential as complementary agents in oral cancer management.

Keywords: Betanin, Health, Disease, Medicine, Illness, Cancer, Oral squamous cell carcinoma.

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INTRODUCTION

Apoptosis, often referred to as programmed cell death, plays an essential role in maintaining the body's internal balance. It is responsible for removing damaged or unwanted cells, supporting immune defense, as often observed in cancer, cells evade death signals, leading to uncontrolled growth and Tumor development. Understanding the molecular mechanism of apoptosis and identifying agents that can restore apoptotic sensitivity in cancer cells is very crucial for effective anticancer therapy (Waring *et al.*, 1991). In this context, bioactive phytochemicals such as betanin and resveratrol have attracted considerable attention for their ability to modulate apoptotic pathways in various cancer models (Hirad *et al.*, 2025).

The water-soluble nitrogen-containing betalain chemical betanin, a naturally occurring pigment from *Beta vulgaris* (beetroot), is distinguished by its vivid red hue and significant antioxidant properties. This compound demonstrates a wide range of biological effects, including anti-inflammatory, hepatoprotective, and anti-cancer actions (Waring *et al.*, 1991; Hirad *et al.*, 2025). Experimental data from *in vitro* and *in vivo* studies suggest that beta in promotes apoptosis in malignant cells by influencing Reactive Oxygen Species (ROS) production, affecting mitochondrial activity, and altering the regulation of apoptosis-related gene expression. Resveratrol, a polyphenolic molecule that is mostly present in peanuts, berries, and grapes, has also shown promise as a chemopreventive and chemotherapeutic drug. By triggering cell cycle arrest, inhibiting angiogenesis, and triggering

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both intrinsic and extrinsic apoptotic pathways, resveratrol has a variety of anticancer actions (Xu *et al*., 2024).

Two well-known *in vitro* models for researching cancer biology and therapeutic screening are KB cells, which are generated from human oral squamous cell carcinoma, and 143B cells, which are derived from osteosarcoma. These cell lines are perfect models to assess the pro-apoptotic effectiveness of bioactive substances because they exhibit resistance to apoptosis and elevated production of anti-apoptotic proteins. Examining how betanin and resveratrol affect these cell lines offers a way to learn how these natural remedies can get past apoptotic resistance, especially in head and bone cancers where traditional treatments frequently have trouble working because of multidrug resistance and off-target toxicity (Cheriyan *et al*., 2025).

Proteins in the Bcl-2 family play a central role in regulating the mitochondrial(intrinsic) apoptotic pathway. Pro-apoptotic members like Bax promote Mitochondrial Outer Membrane Permeabilization (MOMP), resulting in cytochrome c release. This event initiates the cascade activation of executioner caspase such as caspase-9 and caspase-3 (Jayaraman *et al*., 2025). Conversely, anti-apoptotic members like Bcl-2 work to maintain mitochondrial membrane integrity, thereby inhibiting apoptosis. Alterations in the balance of pro- and anti-apoptotic gene expression often triggered by cellular stress or DNA damage can influence this pathway. A key player in this balance is p53, a tumour suppressor gene that, even activated, up regulates Bax and down regulates Bcl-2, pushing cells towards apoptosis (Sebastian *et al*., 2025).

A class of cysteine proteases known as caspases is essential to the apoptotic execution phase. While caspase-3 is the primary executioner caspase, cleaving several structural and repair proteins to cause apoptotic morphological alterations, caspase-9 functions as an initiating caspase that is triggered upon the formation of the apoptosome complex. This study evaluated apoptotic induction by measuring gene expression and enzymatic activity of caspase-3 and caspase-9 using qPCR and Fluor metric assays for comprehensive validation (Payra *et al*., 2024).

Prior reports indicate that betanin can modify expression levels of Bax, Bcl-2, and caspase-9, while simultaneously promoting oxidative stress and disrupting mitochondrial function in lung, breast, prostate cancer models. In many preclinical models, resveratrol has also demonstrated effectiveness by increasing p53-mediated apoptosis, blocking NF-κB signaling, and making cancer cells more sensitive to chemotherapeutic drugs. The combined or separate effects of resveratrol and betanin on oral and bone cancer cell lines, especially with regard to intrinsic apoptotic gene expression and caspase activity, have not, however, been extensively studied. By methodically examining their apoptotic regulation in KB and 143B cells using both molecular and biochemical techniques, this study fills this gap (Jayaraman *et al*., 2025; Morinaga *et al*., 2025).

Cell viability and the cytotoxic concentration thresholds for resveratrol and betanin were assessed using the MTT test. The principal readout for evaluating compound-induced growth suppression is this colorimetric assay, which is based on mitochondrial metabolic activity. Following the profiling, cells were exposed to optimal concentrations of resveratrol and betanin. The expression levels of p53, Bax, Bcl-2, Caspase-3, Caspase -9 mRNA were quantified using real-time PCR, allowing for an in-depth analysis of transcriptional regulation linked to apoptosis. As a result, the transcriptional regulation of apoptosis could be thoroughly examined (Senthil *et al*., 2025; Thoke *et al*., 2025). The use of triplicate biological replicates, which guarantees statistical robustness and reproducibility of the reported effects, is another advantage of the current methodology. In order to capture both early and late apoptotic events, treatments were given at IC_{50} and sub-IC_{50} doses determined from MTT data and all experiments were meticulously controlled using untreated and vehicle-treated cells (Payra *et al*., 2024; Xu *et al*., 2024).

In summary, this research explores how resveratrol and beta in influence intrinsic apoptosis in KB and 143B cell models, particularly through their effects on Caspase activity and gene expression of apoptotic markers like p53, Bax,Bcl-2, caspase-3, caspase-9.This work not only confirms the anticancer efficacy of these natural chemicals but also advances the development of adjunct medicines that can overcome apoptotic resistance in bone and oral malignancies by clarifying their effects on intrinsic apoptosis. It is anticipated that the results will be useful in developing new dietary or adjuvant treatments that reduce overall toxicity and promote tumor-specific apoptosis (Krishnan *et al*., 2025).

MATERIALS AND METHODS

Chemicals and Reagents

We used only high-quality, analytical grade reagents from reliable sources. Betanin (≥98% purity, Sigma-Aldrich, USA) was stored at 4°C in a dry condition and freshly dissolved in sterile distilled water before experiments. Trans-resveratrol (≥99% purity, Cayman Chemical, USA) was prepared as a 100 mM stock in DMSO, stored at −20°C, and diluted freshly so that the final DMSO concentration in cultures never exceeded 0.1%. Cell viability was measured using the MTT assay (Sigma-Aldrich). RNA isolation (TRIzol) and cDNA synthesis (High-Capacity kit, Thermo Fisher) were followed by qRT-PCR with SYBR Green Master Mix (Applied Biosystems). Caspase-3 and caspase-9 activities were assayed using fluorometric kits (Abcam, UK). For cell culture, DMEM, FBS, trypsin-EDTA, and antibiotics were obtained from Gibco, while PBS and other routine reagents were from HiMedia (India). All steps were carried out under sterile conditions according to standard biosafety and culture practices.

Preparation of Stock Solutions

Sterile stock solutions of the test compounds were prepared to ensure consistency and reproducibility. Betanin was accurately weighed, dissolved in sterile distilled water to make a 10 mM stock, vortexed, filtered through a 0.22 µm syringe filter, and stored at 4°C for up to one week. Resveratrol was dissolved in analytical grade DMSO to obtain a 100 mM stock, vortexed until fully dissolved, aliquoted to avoid repeated freeze–thaw cycles, and stored at −20°C. The final DMSO concentration in treatments was always kept below 0.1%, confirmed to be non-toxic to KB and 143B cells. For experiments, working dilutions were freshly prepared in culture medium. The MTT reagent was filtered, diluted in sterile PBS, and stored at 4°C in the dark. TRIzol, cDNA synthesis reagents, and SYBR Green master mix were used as per manufacturer's instructions for gene expression studies. All working solutions were freshly prepared before each experiment to maintain accuracy and activity (Taneesha *et al*., 2025).

Cell Culture and conditions

Human osteosarcoma (143B) and oral squamous carcinoma (KB) cells were procured from National Centre for Cell Science, Pune and routinely cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin. Cells were maintained in 75 cm² flasks at 37°C in a humidified 5% CO_2 atmosphere, with medium changes every 2–3 days. Subculturing was performed using 0.25% trypsin-EDTA once cultures reached 70–80% confluency. For experiments, cells were seeded into 96-well or 6-well plates, and only logarithmically growing cells at passages 5–20 were used to ensure consistent responses. All work was conducted under sterile conditions in a biosafety cabinet, and cell morphology and confluence were routinely assessed using an inverted phase-contrast microscope (Taneesha *et al*., 2025).

MTT Assay for Cell Viability Analysis

The MTT assay was used to evaluate the cytotoxic effects of betanin and resveratrol on KB and 143B cells. To enable adhesion, cells were planted in 96-well plates at a density of 5×10^4 cells/well and incubated for the whole night. B10 and B25 were treated with 10 µM and 25 µM betanin, respectively; R10 and R25 were treated with 10 µM and 25 µM resveratrol; a combination group (B+R) was treated with 10 µM of both betanin and resveratrol; and Negative Control (NC) was treated with untreated cells and Vehicle Control (VC) was treated with 0.1% DMSO. Cells were incubated for 24 hr after treatments were administered in triplicate. Following treatment, each well received 20 µL of MTT solution (5 mg/mL in PBS), which was then incubated for four hours at 37°C. 100 µL of DMSO was used to dissolve the resultant formazan crystals, and a microplate reader was used to quantify absorbance at 570 nm. To assess dose-dependent and synergistic effects, statistical comparisons between treatment groups were performed, and cell viability was expressed as a percentage compared to the negative control group (Rajasekar *et al*., 2024).

Gene Expression Analysis by qRT-PCR

Since this was a pilot study, gene expression analysis was carried out only in KB oral carcinoma cells. After 24 hr of treatment exposure, total RNA was isolated using TRIzol reagent (Thermo Fisher Scientific, USA) according to the manufacturer's protocol, and its concentration and purity were determined with a Nanodrop spectrophotometer. One microgram of RNA was reverse-transcribed into cDNA using the High-Capacity cDNA Reverse Transcription Kit. Quantitative PCR was performed with SYBR Green Master Mix (Applied Biosystems) using gene-specific primers for p53, Bax, Bcl-2, caspase-3, and caspase-9, with GAPDH as the internal control. The cycling conditions included an initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method, normalized to GAPDH, and expressed as fold change compared with untreated controls. All reactions were performed in triplicate to ensure accuracy and reproducibility (Marunganathan *et al*., 2024).

RESULTS

Cell Morphology of KB Cells

When betanin, resveratrol, and their combination were applied to KB cells, phase-contrast microscopy analysis showed clear morphological changes. The KB cells in the Negative Control (NC) group showed the normal epithelial morphology of healthy proliferating cells, including polygonal shape, intact cell membranes, and intense cell-to-cell adhesion (Figure 1). When compared to the NC group, the Vehicle Control (VC) group treated with 0.1% DMSO did not exhibit any appreciable morphological changes. Small cellular shrinkage and decreased confluency were observed in cells treated with betanin at 10 µM (B10), but rounding, detachment, and membrane blebbing were prominent in cells treated with 25 µM (B25), indicating early apoptotic characteristics. Both R10 and R25, the resveratrol-treated groups, displayed dose-dependent morphological disruption; R25's cytoplasmic condensation and cell fragmentation were particularly noticeable. The combination group (B+R) notably showed the most severe morphological alterations, which were consistent with late-stage apoptosis and included significant membrane rupture, cellular shrinkage, and separation from the substrate. These findings lend credence to the cytotoxic and pro-apoptotic actions of resveratrol and betanin, especially when combined, on oral cancer cells (Figure 1).

Cell Morphology of 143B cells

Morphological assessment of 143B osteosarcoma cells following treatment revealed characteristic apoptotic changes, varying by concentration and compound. In the Negative Control (NC)

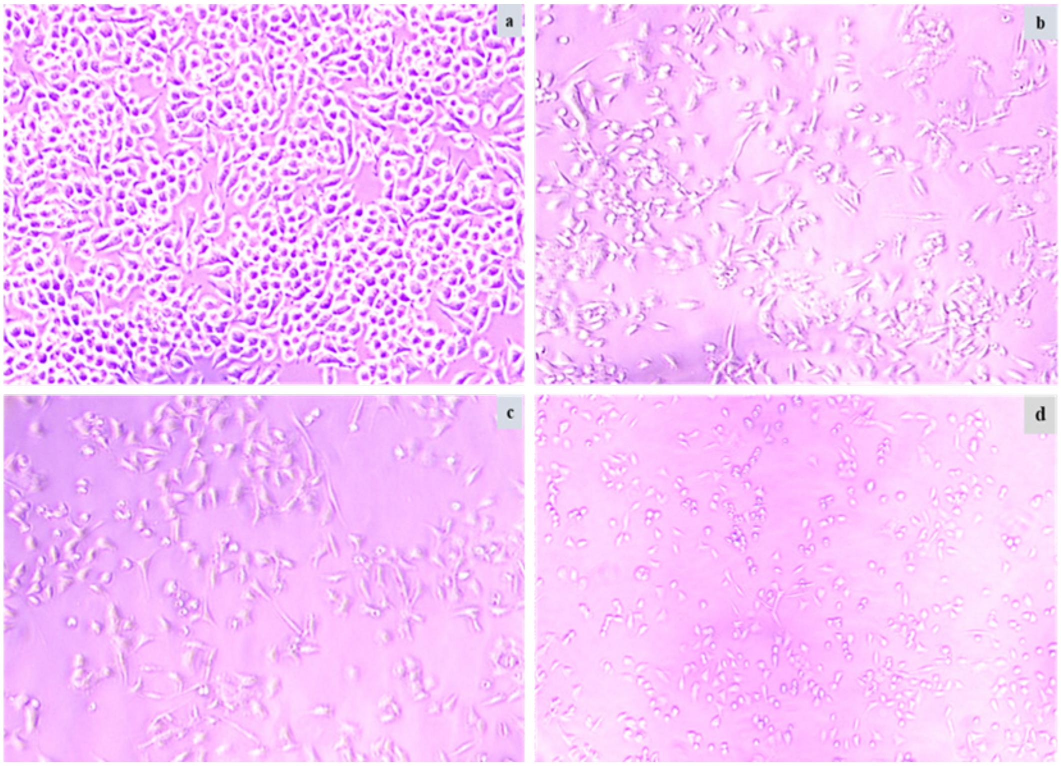

Figure 1: Morphological changes of KB cells treatment with betanin and resveratrol (24 hr). (a) Negative Control (untreated) showing normal epithelial morphology with intact cell–cell contacts and polygonal shape. (b) Betanin 25 µM: cells exhibit rounding, shrinkage, and partial detachment indicating apoptotic features. (c) Resveratrol 25 µM: pronounced cytoplasmic condensation and membrane blebbing observed. (d) Combination of Betanin + Resveratrol (10 µM each): extensive cell shrinkage, fragmentation, and detachment suggestive of advanced apoptosis. Images captured under phase-contrast microscopy at 200× magnification.

group, 143B cells exhibited a typical spindle-shaped fibroblastic morphology with elongated cytoplasm and firm adherence to the culture surface (Figure 2). The cells appeared healthy and densely packed, forming uniform monolayers. Treatment with betanin at 25 µM (B25) led to noticeable cellular shrinkage, membrane irregularities, and partial detachment from the substrate. Similarly, resveratrol at 25 µM (R25) caused cytoplasmic condensation, membrane blebbing, and decreased cell density, suggestive of apoptosis. The most noticeable apoptotic characteristics, such as severe shrinkage, fragmented cells, and extensive detachment, were displayed by the combination group (B+R, 10 µM each), suggesting increased cytotoxicity through synergistic interaction. These morphological alterations in 143B cells provide credence to the pro-apoptotic effects of resveratrol and betanin, particularly when combined (Figure 2).

MTT Assay Results in KB and 143B Cells

Treatment with betanin, resveratrol, and their combination resulted in a measurable, dose-responsive reduction in cell viability as determined by the MTT assay in both KB oral cancer cells (Figure 3) and 143B osteosarcoma cells (Figure 4). In comparison to the negative control, betanin treatment at 10 µM (B10) and 25 µM (B25) decreased cell viability in KB cells to roughly 82% and 64%, respectively. Viability decreased more noticeably at 10 µM (R10) and 25 µM (R25) of resveratrol, falling to 78% and 58%, respectively. With viability dropping to about 45%, the combined treatment (B+R, 10 µM each) produced the biggest drop, suggesting a possible synergistic impact. Similarly, in 143B cells, B10 and B25 treatments reduced viability to around 85% and 66%, while R10 and R25 showed reductions to 80% and 60%, respectively. The B+R combination further decreased viability to approximately 48%, aligning with the observations in KB cells. These findings suggest that both compounds exert cytotoxic effects individually, but their combination significantly enhances anticancer efficacy through potential synergistic mechanisms in both epithelial and mesenchymal cancer cell models.

Gene Expression Analysis of Apoptotic Markers in KB Cells

Quantitative real-time PCR analysis of KB oral cancer cells following 24-hr treatment with betanin, resveratrol, and their combination revealed notable alterations in the expression of key genes associated with apoptosis. The tumor suppressor p53 was significantly upregulated, showing a 1.8-fold increase with 25 µM betanin, a 2.1-fold increase with 25 µM resveratrol, and a marked 2.7-fold rise in the combination group (10 µM each), suggesting enhanced activation of DNA damage response pathways and apoptotic signaling. The anti-apoptotic gene Bcl-2 was downregulated across all treated groups, with the combination therapy resulting in the most substantial suppression (0.4-fold relative to control). In contrast, the pro-apoptotic gene Bax exhibited a dose-dependent rise in expression, with the combination treatment yielding the highest increase (3.0-fold). Additionally, mRNA levels of caspase-3 and caspase-9 were elevated in all experimental groups, with the combination treatment again producing the greatest fold change 2.9 and 2.6, respectively indicating robust activation of the intrinsic (mitochondrial) apoptotic cascade. Collectively, these results suggest that co-treatment with betanin and resveratrol exerts a synergistic pro-apoptotic effect in KB cells by modulating the transcription of multiple apoptosis-regulating genes (Figure 5).

Gene expression levels were normalized against GAPDH and analyzed using the $2^{-\Delta\Delta Ct}$ method. The combination group demonstrated the most pronounced upregulation of pro-apoptotic markers and significant suppression of Bcl-2, reflecting intensified activation of the mitochondrial apoptotic pathway. Values are expressed as Mean ± SD ($n = 3$). A p-value below 0.05 was considered statistically significant in comparison to the negative control.

DISCUSSION

Affecting the head and neck more than most other cancers, oral squamous cell carcinoma is marked by its rapid local progression and poor response to routine treatment strategies. The evasion of apoptosis, which permits tumor cells to persist in spite of oncogenic stress and therapeutic interventions, is one of the main characteristics of OSCC growth. Therefore, a viable approach to oral cancer treatment is to target apoptotic resistance with safe and efficient medicines. In our study, we looked at the pro-apoptotic effects of resveratrol and betanin, two natural substances, on KB cells, a commonly utilized OSCC cell line. Our results show that both substances cause apoptosis on their own, but when combined, they boost apoptotic signaling in a synergistic way, as shown by morphological alterations, decreased viability, caspase activation, and changed expression of genes linked to apoptosis (Zhao *et al.*, 2025).

The MTT assay results showed a clear dose-dependent reduction in KB cell viability upon exposure to betanin and resveratrol. While both agents individually reduced cell viability significantly, the combination treatment (10 μM each) resulted in the most profound decrease, suggesting a potential synergistic cytotoxic effect. This observation is consistent with previous studies

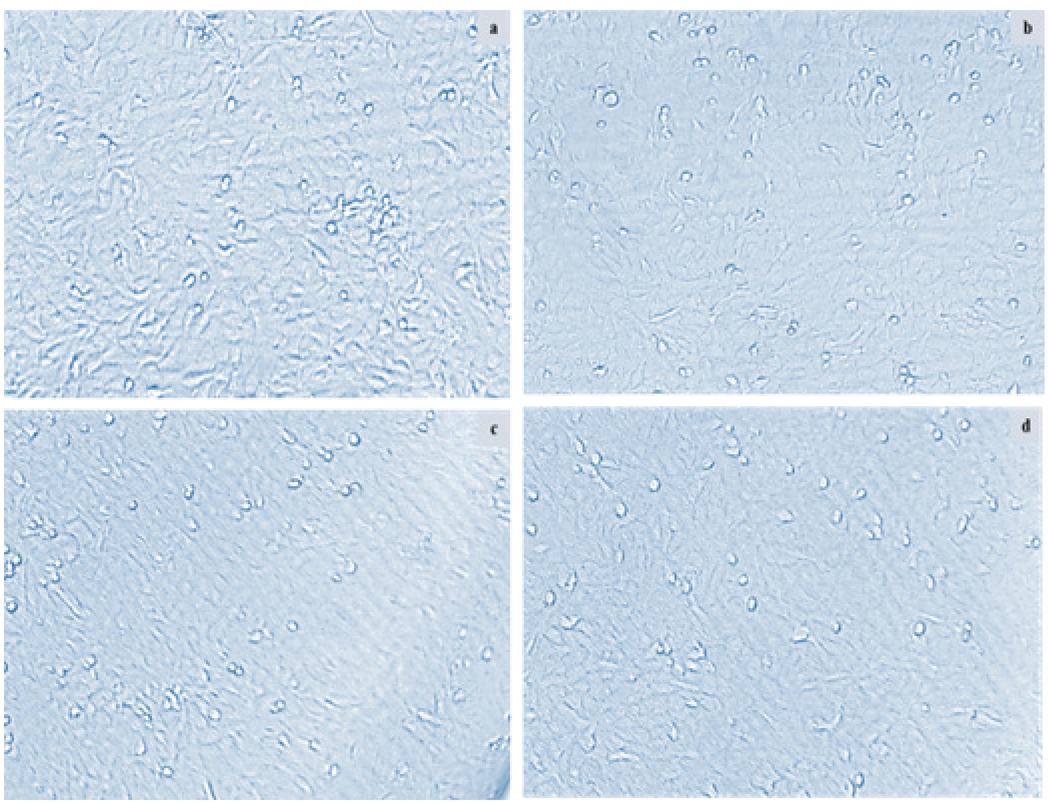

Figure 2: Morphological changes in 143-B cells treatment with betanin and resveratrol (24 hr). (a) Negative Control (untreated): spindle-shaped morphology with healthy cell monolayer. (b) Betanin 25 μM: cells display shrinkage, membrane irregularities, and partial detachment. (c) Resveratrol 25 μM: cells exhibit cytoplasmic condensation and membrane blebbing. (d) Combination of Betanin + Resveratrol (10 μM each): pronounced shrinkage, fragmentation, and cell loss indicative of advanced apoptosis. Images were captured under phase-contrast microscopy at 200× magnification.

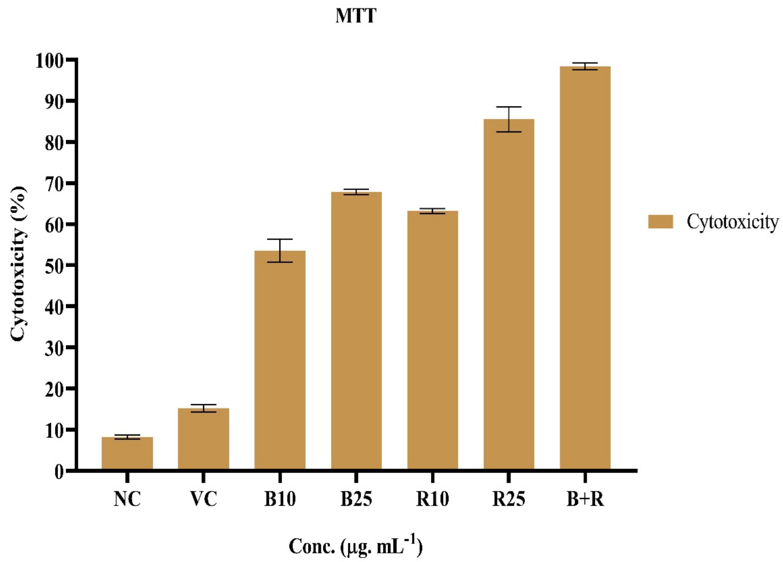

Figure 3: Cytotoxic effect of betanin and resveratrol combination on KB cells (24 hr). MTT assay results showed the percentage of viable KB cells after 24-hr exposure to betanin (10 µM and 25 µM), resveratrol (10 µM and 25 µM), and their combination (10 µM each). The combination group (B+R) caused the strongest reduction in viability, indicating a synergistic cytotoxic effect. Data are expressed as Mean ± Standard Deviation (*n* = 3), and differences with $p < 0.05$ were considered statistically significant compared to the untreated control.

reporting that combining bioactive compounds can yield enhanced anticancer effects by targeting multiple signaling pathways simultaneously. Notably, the doses used in this study were relatively low, reinforcing the potential of using combination strategies to achieve higher efficacy with minimal toxicity (Bisht *et al*., 2025).

The cytotoxic and pro-apoptotic effects were further validated by morphological analysis of KB cells after treatment. Strong cell–cell adhesion and a characteristic epithelial morphology were maintained by control cells. On the other hand, cells treated with resveratrol and betanin, particularly together, showed the telltale signs of apoptosis, such as rounding, membrane blebbing, cell shrinkage, and separation from the substrate. These alterations are consistent with the morphological characteristics of cells going through programmed cell death and are suggestive of irreversible cellular damage (Dhar *et al*., 2025).

To gain insight into the mechanism driving the cytotoxic response, we investigated the expression of genes central to the intrinsic apoptotic pathway, which is regulated through mitochondrial signaling. This process is tightly controlled by members of the Bcl-2 protein family, who govern how cells respond to intracellular stress. According to our qRT-PCR data, Bcl-2 was strongly downregulated and p53 and Bax were significantly upregulated, especially in the combination group. The well-known tumor suppressor p53 is essential for encouraging apoptosis in reaction to cellular stress and DNA damage. When it is activated, it increases the transcription of Bax, a pro-apoptotic protein that facilitates the permeabilization of the mitochondrial membrane, which releases cytochrome c and activates caspases downstream. The pro-apoptotic response is amplified by the simultaneous reduction in Bcl-2 expression, an important survival protein of the Bcl-2 family. These chemical alterations clearly imply that the betanin-resveratrol combination damages mitochondrial integrity, which in turn causes apoptosis.

We found that caspase-9 and caspase-3 were significantly upregulated at the mRNA level, which was corroborated by enhanced enzymatic activity as determined by RFU-based fluorometric assays. When the apoptosome complex forms after cytochrome c release, caspase-9 is activated. This in turn triggers caspase-3, the main executioner caspase that cleaves cellular substrates and forms apoptotic bodies. The successful activation of the intrinsic apoptotic machinery is indicated by the elevated activity of both caspases in treated cells, especially in the combination group. Notably, fluorometric data match gene expression results, providing functional support to the transcriptional findings and demonstrating that apoptosis, not necrosis or other types of cell death, is the mechanism underlying the observed cytotoxicity.

Because of their complementary modes of action, betanin and resveratrol have been shown to work in concert. Betanin is well recognized for producing Reactive Oxygen Species (ROS), which can cause mitochondrial stress and trigger apoptosis. Conversely, it has been demonstrated that resveratrol alters a number of signaling cascades, including as p53 activation, NF-κB inhibition, and direct contact with apoptotic proteins. Together, these substances have the potential to increase oxidative stress and apoptotic signaling while circumventing defense systems frequently deployed by cancer cells (Senthil *et al*., 2022).

In a variety of cancer models, prior research has documented comparable synergistic effects of phytochemicals and polyphenols.

For instance, curcumin has been proven to have stronger effects on prostate, colon, and breast cancer cells when combined with EGCG or resveratrol. Our results show the therapeutic potential of combining betanin and resveratrol for improved efficacy in the treatment of oral cancer and extend this idea to OSCC. Crucially, this tactic might aid in lowering each compound's effective dosage, reducing the possibility of off-target effects and boosting clinical safety.

These findings also reinforce the expanding scientific consensus that bioactive molecules from plants can serve as valuable

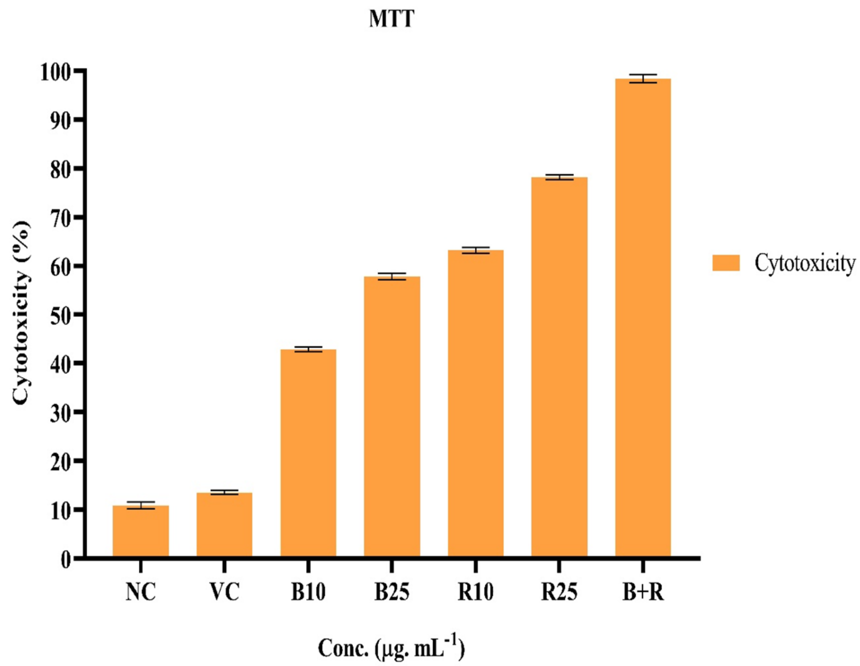

Figure 4: Cytotoxic effect of betanin and resveratrol combination on 143B cells (24 hr). MTT assay results showed the percentage of viable 143B cells after 24-hr treatment with betanin (10 μM and 25 μM), resveratrol (10 μM and 25 μM), and their combination (10 μM each).

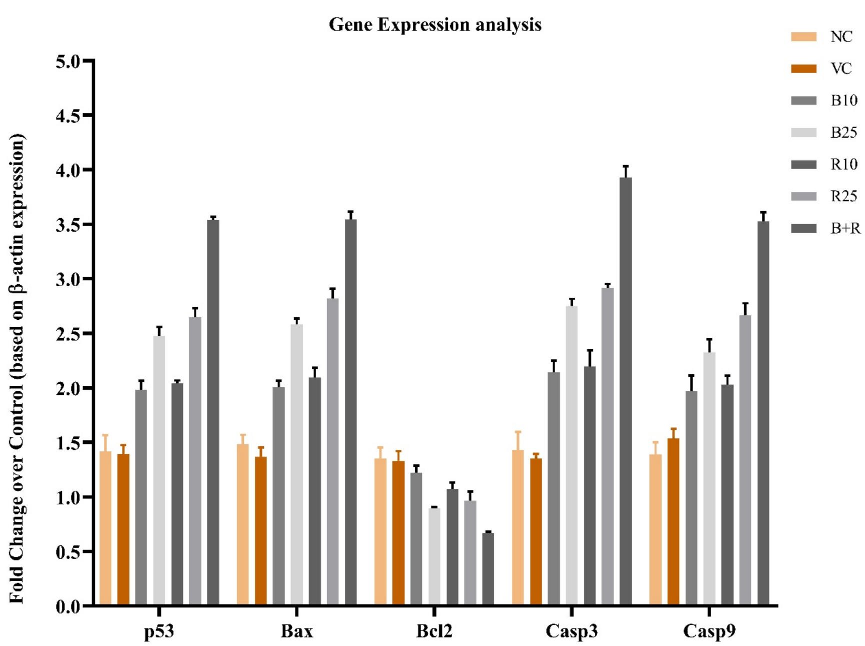

Figure 5: Relative expression of apoptosis-related genes (p53, Bax, Bcl-2, caspase-3, and caspase-9) in KB cells following 24-hr exposure to betanin (10 μM and 25 μM), resveratrol (10 μM and 25 μM), and their combined treatment (10 μM each). Gene expression levels were normalized against GAPDH and analyzed using the 2^−ΔΔCt method. The combination group demonstrated the most pronounced upregulation of pro-apoptotic markers and significant suppression of Bcl-2, reflecting intensified activation of the mitochondrial apoptotic pathway. Values are expressed as Mean ± SD ($n = 3$). A p-value below 0.05 was considered statistically significant in comparison to the negative control.

adjuncts in cancer treatment strategies. They are appealing substitutes or supplements to synthetic chemotherapeutics, which are frequently constrained by toxicity and resistance, due to their capacity to specifically target cancer cells, modify gene expression, and induce apoptosis. The use of betanin and resveratrol in the treatment of oral cancer has translational potential because they are both naturally occurring substances that have shown safety in preclinical and clinical settings.

The use of a single cancer cell line (KB) without comparison to normal oral epithelial cells is one of the study's limitations. Future research could more precisely evaluate the safety and selectivity of these substances by incorporating non-cancerous control cells. Furthermore, greater research examining the effects of this combination *in vivo* or in 3D models of oral cancer would offer a more thorough understanding of its pharmacokinetic characteristics and therapeutic potential. Studying how they interact with important survival pathways like PI3K/Akt, MAPK, or NF-κB may also reveal mechanistic details that go beyond apoptosis (Saber *et al.*, 2023).

To sum up, the present study offers strong evidence that resveratrol and betanin both cause apoptosis in KB oral cancer cells on their own, and that they work in concert to increase pro-apoptotic signaling. Activation of the mitochondrial apoptotic pathway is suggested by increased expression of p53, Bax, caspase-9, and caspase-3, coupled with reduced Bcl-2 levels and heightened caspase enzymatic activity. These results highlight the therapeutic value of natural chemical combinations and encourage more research into the synergy between betanin and resveratrol as a possible treatment approach for oral cancer (Zhou *et al.*, 2005).

CONCLUSION

This study provides evidence that betanin and resveratrol induce apoptosis in KB oral cancer cells through activation of the intrinsic mitochondrial pathway. Both compounds suppressed the expression of the anti-apoptotic gene Bcl-2, while simultaneously enhancing the expression of key pro-apoptotic markers such as p53**,** Bax**,** caspase-3, and caspase-9. Notably, the combined treatment elicited the strongest apoptotic response and most significant reduction in cell viability, indicating a synergistic therapeutic effect. These results call for greater research in preclinical and clinical settings and demonstrate the promise of natural bioactive combinations as a safer and more successful treatment strategy for oral cancer.

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ABBREVIATIONS

Apaf-1: Apoptotic protease activating factor-1; **Bax:** Bcl-2-associated X protein; **Bcl-2:** B-cell lymphoma 2; **DMEM:** Dulbecco's Modified Eagle Medium; **IC_{50}:** Half-maximal inhibitory concentration; **KB cells:** Human oral squamous carcinoma cell line; **143B cells:** Human osteosarcoma cell line; **MOMP:** Mitochondrial outer membrane permeabilization; **MTT:** 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **NF-κB:** Nuclear factor kappa-light-chain-enhancer of activated B cells; **PCR:** Polymerase chain reaction; **qPCR / RT-qPCR:** Quantitative real-time polymerase chain reaction; **ROS:** Reactive oxygen species; **p53:** Tumor protein p53; **mRNA:** Messenger ribonucleic acid.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Mampi Payra: Conceptualization, methodology, experimental work, data curation, formal analysis, and manuscript drafting. **Taniya Mary Martin:** Validation, experimental supervision, data interpretation, and critical manuscript revision. **Meenakshi Sundaram Kishore Kumar:** Project administration, resource provision, overall supervision, manuscript review, and final approval. **Jayalakshmi Somasundaram:** Assistance in experimental methodology, data acquisition, and technical support. **Pallabi Ghosh:** Literature review, data compilation, visualization, and proofreading of the manuscript.

SUMMARY

Betanin and resveratrol effectively triggered apoptosis in KB oral cancer cells by downregulating Bcl-2 and upregulating p53, Bax, caspase-3, and caspase-9, these activity confirmed the activation of the intrinsic mitochondrial pathway. The combined treatment showed the strongest apoptotic effect and greatest reduction in cell viability, highlighting a synergistic action. These findings support the potential of natural bioactive combinations as promising and safer therapeutic strategies for oral cancer.

REFERENCES

Bisht, A., Avinash, D., Sahu, K. K., Patel, P., Das Gupta, G., & Kurmi, B. D. (2025). A comprehensive review on doxorubicin: mechanisms, toxicity, clinical trials, combination therapies and nanoformulations in breast cancer. *Drug Delivery and Translational Research*, *15*(1), 102-133.

Cheriyan, B. V., Srinivasan, P., Jayaraj, G., Karunakar, K. K., Sainath, P. B., Shanmugam, A., … & Elumalai, K. (2025). *In silico* and *in vitro* Evaluation of the Cytotoxic Potential of Hinokitiol against Osteosarcoma by Targeting Glycogen Synthase Kinase 3β. *Turkish Journal of Pharmaceutical Sciences*, *21*(6), 499.

Dhar, K., Jena, K. K., Mehto, S., Sahu, R., Murmu, K. C., Mahajan, A. A., … & Chauhan, S. (2025). Programmed cell revival from imminent cell death enhances tissue repair and regeneration. *The EMBO Journal*, *44*(19), 5244-5289.

Hirad, A. H., Alarfaj, A. A., Ravindran, B., & Narasimhamoorthi, S. P. (2025). Betanin inspired zinc oxide nanoparticles: The potential antioxidant and anticancer activity against human lung cancer cell line (A549). *Biochemical and Biophysical Research Communications*, *742*, 151019.

Jayaraman, S., & Veeraraghavan, V. P. (2025). Deoxyelephantopin induces cell death in oral cancer cells via the downregulation of AKT1-mTOR-mediated mechanisms. *Journal of Oral and Maxillofacial Pathology*, *29*(2), 193-205.

Krishnan, R. P., Pandiar, D., Dilipan, E., Inbarajan, A., Ramani, P., & Jayaraman, S. (2025). Role of PDGFRA-Associated miR-140 in Modulating Necroptotic Proteins in Oral Squamous Cell Carcinoma: A Molecular Docking Study. *Journal of International Oral Health*, *17*(1), 73-81.

Marunganathan, V., Kumar, M. S. K., Kari, Z. A., Giri, J., Shaik, M. R., Shaik, B., & Guru, A. (2024). Marine-derived κ-carrageenan-coated zinc oxide nanoparticles for targeted drug delivery and apoptosis induction in oral cancer. Molecular biology reports, 51(1), 89.

Morinaga, S., Han, Q., Mizuta, K., Kang, B. M., Bouvet, M., Yamamoto, N., … & Hoffman, R. M. (2025). Highly synergistic eradication of 143B osteosarcoma cells *in vitro* by the combination of recombinant methioninase, chloroquine, and rapamycin targeting methionine addiction, autophagy, and mTOR, respectively. *Anticancer Research*, *45*(3), 935-941.

Rajasekar, N., Mohanraj, K. G., & Martin, T. M. (2024). Advanced dental care: β-chitosan zinc oxide nanoparticles targeting cariogenic microorganisms. *Cureus*, *16*(8).

Saber, A., Abedimanesh, N., Somi, M. H., Khosroushahi, A. Y., & Moradi, S. (2023). Anticancer properties of red beetroot hydro-alcoholic extract and its main constituent; betanin on colorectal cancer cell lines. *BMC Complementary Medicine and Therapies*, *23*(1), 246.

Sebastian, S., Martin, T. M., & Kumar, M. S. K. (2025). Thymoquinone-Loaded Zinc Nanoparticles Mitigate Inflammation and Inhibit Glioblastoma Progression: A Novel Therapeutic Approach. *Pharmacognosy Research 17(3)*:850-858.

Senthil, R. (2025). Bevacizumab-conjugated curcumin nanoparticles promote cytotoxicity and apoptosis in human malignant oral keratinocytes *in vitro*. *Journal of Oral and Maxillofacial Surgery*. *83(9)*, 1170-1178.

Senthil, R., Sundaram, K. K. M., Bupesh, G., Usha, S., & Saravanan, K. M. (2022). Identification of oxazolo [4, 5-g] quinazolin-2 (1H)-one Derivatives as EGFR Inhibitors for Cancer Prevention. *Asian Pacific Journal of Cancer Prevention: APJCP*, *23*(5), 1687.

Soundharya, M., Subasree, S., Ramya, R., & Pradeep Kumar, Y. (2025). Gated Graph Sequence Neural Network-Based Prediction of Drug-Gene Association for Nucleoside Proteins in Oral Cancer. *South Eastern European Journal of Public Health*, S2,2197-5248.

Taneesha S., Karthik Ganesh M., Taniya Mary M., (2025) Exploring Betanin's antiangiogenic and Prohealing effects in Dental tissue regeneration evidence from zebrafish and CAM models. *Bulletin of Stomatology and Maxillofacial Surgery*. *21(9)*.227-236.

Thoke, G. M., Sinduja, P., Kaliaperumal, K., Gandham, S., Rajasekar, S., & Murugesan, P. (2025). Anticancer Effect of Ascidian *Aplidium* Species Extract against Ovarian and Breast Cancer Cell Lines. *Journal of Pharmacy and Bioallied Sciences*, *17*(Suppl 2), S1839-S1841.

Waring, P., Kos, F. J., & Müllbacher, A. (1991). Apoptosis or programmed cell death. *Medicinal research reviews*, *11*(2), 219-236.

Xu, X., Zhang, D., Liu, X., Zheng, R., & Jiang, T. (2024). Ultrasonic-Assisted Extraction and Antioxidant Evaluation of Resveratrol from Peanut Sprouts. *Processes*, *12*(10), 2295.

Zhao, C. (2025). Exploring cell death pathways in oral cancer: mechanisms, therapeutic strategies, and future perspectives. *Discover Oncology*, *16*(1), 395.

Zhou, H. B., Chen, J. J., Wang, W. X., Cai, J. T., & Du, Q. (2005). Anticancer activity of resveratrol on implanted human primary gastric carcinoma cells in nude mice. *World Journal of Gastroenterology: WJG*, *11*(2), 280.

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