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Synergistic Effects of Betanin and Theobromine on the PI3K/AKT/mTOR Pathway in KB Cells: A Molecular Insight into ROS-Mediated Apoptosis

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ABSTRACT

Background: Oral squamous cell carcinoma (OSCC) is a common cancer that has a high recurrence rate and little response to treatment. Natural substances that alter survival and apoptotic pathways, like betanin and theobromine, have promising anticancer effects. This study examines the combined cytotoxic effects of betanin and theobromine on KB oral cancer cells, with a particular emphasis on the production of reactive oxygen species (ROS) and the modulation of the PI3K/AKT/mTOR pathway.

Methods: KB cells were treated with varying concentrations of betanin (1–100 µg/mL), theobromine (1–100 µg/mL), and their combinations (Combo_IC20, Combo_IC50, Combo_IC70). Cell viability was assessed using the MTT assay. Using DCFH-DA staining and fluorescence intensity, intracellular ROS levels were determined. Following a 24-hour treatment, the expression of PIK3CA, AKT1, mTOR, BAX, BCL2, and CASP3 was examined using quantitative real-time PCR (qRT-PCR).

Results: Both betanin and theobromine showed dose-dependent cytotoxicity, with combination treatments—especially Combo_IC50—exhibiting significantly enhanced cell death. ROS levels increased substantially in all treated groups, with the highest levels observed in the combination groups, indicating oxidative stress-mediated apoptosis. Gene expression

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analysis revealed significant downregulation of PIK3CA, AKT1, and mTOR in combination groups, along with upregulation of BAX and CASP3, and downregulation of anti-apoptotic BCL2, confirming mitochondrial apoptotic pathway activation.

Conclusion: Betanin and theobromine act synergistically to inhibit KB cell proliferation by inducing oxidative stress and suppressing the PI3K/AKT/mTOR pathway, leading to apoptosis. These findings support the potential application of this phytochemical combination as a safe and effective strategy for oral cancer therapy.

Keywords: *Betanin, Theobromine, Oral cancer, PI3K/AKT/mTOR, ROS, Apoptosis, KB cells, Synergistic effect.*

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1. INTRODUCTION

Oral squamous cell carcinoma (OSCC) is a severe health concern around the world, particularly in head and neck cancers. It is extremely challenging to control since it grows quickly, recurs frequently, and is tough to treat. With over 90% of all cases being OSCC, it is the most prevalent kind of oral cancer. Treatment has a lower chance of success because it is frequently discovered at a late stage. Although radiation, chemotherapy, and surgery have improved, the five-year survival rate remains extremely low. This is due to the fact that oral cancer requires better, safer, and more accurate therapies since cancer cells frequently develop resistance to therapy and reappear.

First, they use KB cells originating from human epidermoid carcinoma in vitro, to study oral cancer induction processes and to try to find new therapies. While phytochemicals—natural compounds from plants-have been attracting significant interest recently, what makes these substances interesting is that they interact with various cancer-associated signals but are also relatively low in toxicity to normal cells. Some classical examples are sulforaphane, quercetin, and curcumin. Research has indicated that these substances are able to mediate oxidative stress and cell death, particularly via the PI3K/AKT/mTOR pathway. (Kim and Kim 2018) (Ju et al. 2024) (Xie et al. 2021)(Aditya et al. 2021).

Research has aimed at compounds like betanin and theobromine because of their phytochemicals with known antioxidant and anticarcinogenic characteristics. Betanin is famous for its cancer preventive properties and was shown to reduce inflammation, protect the liver, and prevent free radical damage to cells (Liu et al. 2023). Its effect is mainly achieved by lowering the level of state of reactive oxygen species (ROS) that damage mitochondria and lead to the death of the cell through a number of proteins such as Bax, Bcl-2, and Caspase-3 (Liu et al. 2023) . It also helps to control tumor cell migration and invasion by blocking vital signals required for tumor growth and survival, including the PI3K/AKT/mTOR pathway which is important for cell growth, survival, and metabolism.

More and more people are discovering that Theobromine which is found in cacao can potentially help with heart conditions, and offer protective effects on the brain, and even assist in battling cancer. Unlike caffeine, theobromine works differently on the body since its effects are far milder. In comparison to caffeine, theobromine is much safer, and more effective as a long-term treatment plan. Theobromine also has negative effects such as potentially harming DNA, heightening stress levels in the body, and inhibiting vital cancer pathways like PI3K/AKT and NF-κB. It is possible that these actions would inhibit the growth and survival of cancer cells. (Kim and Kim 2018)

The PI3K/AKT/mTOR pathway is what allows cancer cells to live, grow and not die. PI3K makes PIP3 which in turn activates AKT when this pathway is turned on. Among the many things that happen inside the cell is the activation of mTOR which is important for protein synthesis and cell division. Unfortunately oral squamous cell carcinomas often have a defect in this pathway which results in rapid tumor growth, poor prognosis and resistance to treatment. (Hambright et al. 2015) (Ran et al. 2019)(Devi et al. 2021).New medicines are focusing on this route because of its significant significance in cancer.

Oxidative stress is big in cancer especially in the PI3K/AKT/mTOR pathway. In healthy cells ROS are produced during signaling and metabolism. But cancer cells have excess ROS. This excess can help cancer cells survive by indirectly activating the PI3K/AKT pathway. But if ROS levels get too high they can harm mitochondria and lead to cell death by activating Bax and Caspase-3 (Granato et al.,2016) (Rahman et al. 2021) (Ju et al. 2024). Since ROS can have both positive and negative effects targeting them might be a good strategy for cancer treatment. By lowering ROS levels and inhibiting the PI3K/AKT/mTOR pathway we might be able to tackle cancer cells.

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This is the point at which combining betanin and theobromine becomes intriguing. By attacking the PI3K/AKT/mTOR pathway and the mitochondria of cancer cells, bethanin lowers oxidative stress and destroys the cells (Liu et al. 2023). Another effective strategy to combat cancer is to utilize theobromine to induce oxidative damage in cancer cells and stop them from living. Together, they may be more effective than each one alone. Theobromine increases sensitivity and reduces the ability of cells to survive, while betanin maintains the chemical balance in cells and increases the likelihood that they will die.

Even though each compound on its own shows some good effects, how betanin and theobromine work together against oral squamous cell carcinoma (OSCC)—especially in terms of making more reactive oxygen species (ROS) and messing up the PI3K/AKT/mTOR pathway—has not been well studied. Research on other natural compounds like baicalin, curcumin, and quercetin suggests that raising ROS levels while blocking signals that help cancer cells survive can be a strong way to fight cancer (Pang et al. ,2022) (Ran et al. 2019) (Granato et al.2016).This makes it a good idea to look into mixing different plant chemicals to tackle tough cancers like OSCC.

Thus, this study aims to assess the synergistic anticancer effects of betanin and theobromine in KB oral cancer cells, focusing on their ability to elevate ROS levels and disrupt the PI3K/AKT/mTOR signaling pathway. Our primary hypothesis is that the combined use of these compounds will enhance cytotoxicity, induce mitochondrial damage through ROS accumulation, and influence the expression of key genes related to apoptosis and survival, specifically PIK3CA, AKT1, mTOR, BAX, BCL2, and CASP3.

To explore this topic, we implemented a three-part experimental strategy:
1. We utilized the MTT assay to evaluate cell viability.
2. DCFDA staining was performed to quantify ROS levels within the cells.
3. qRT-PCR was carried out to examine alterations in gene expression related to apoptosis and survival pathways.
This comprehensive approach provided a better understanding of how the combination of betanin and theobromine influences cancer cell behavior at the molecular level. Our main goal is to investigate phytochemical-based therapies that are both effective and less toxic, potentially offering new alternatives to enhance existing oral cancer treatments.
This study fills an important gap in existing research by providing new information on how natural substances such as betanin and theobromine work on their own and together. The findings support the growing field of integrative oncology, where compounds from plants are used carefully in cancer treatments to make therapies more effective, safer, and better for patients.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

We used high-purity betanin (≥98%) and theobromine (≥99%) from Sigma-Aldrich and kept them properly stored to maintain quality. Betanin and theobromine were first mixed in sterile solutions such as PBS or DMSO, based on which solvent worked best. Before each experiment, these mixtures were first diluted with cell culture medium.

KB oral cancer cells grew in RPMI-1640 medium that included fetal bovine serum and antibiotics. The antibiotics used were penicillin, streptomycin, and amphotericin B, all provided by Gibco, which is part of Thermo Fisher.. Trypsin-EDTA and PBS were also from Gibco.

For testing cell survival, we used the MTT reagent and DMSO from HiMedia (India). We used a ROS-sensitive dye (DCFH-DA) from Sigma-Aldrich to measure oxidative stress. RNA was taken out with TRIzol, then changed to cDNA using a reverse transcription kit from Invitrogen. For gene expression, we used SYBR Green Master Mix and primers bought from Eurofins Genomics in India.. All steps followed sterile methods and standard lab practices for reliable results.

2.2 Preparation of Betanin, Theobromine, and Combined Solutions

Fresh betanin and theobromine solutions were prepared before each experiment to keep their quality. Betanin was prepared by dissolving 10 mg in 1 mL of sterile water to make a 10 mg/mL solution. The mixture was stirred until clear and stored at 4°C in amber tubes to protect it from light. Since theobromine does not mix well with water, 10 mg was dissolved in 1 mL of DMSO. The solution was split into smaller portions and kept at −20°C in dark vials.

When both substances were used together, equal amounts from each stock were combined and then diluted in RPMI-1640 medium that contained 10% FBS. This process created final concentrations such as 25, 50, or 100 micrograms per milliliter, or set fixed ratios like 1 to 1, 1 to 2, or 2 to 1. The final DMSO level stayed under 0.1% to avoid harming cells. All solutions were filtered through 0.22 µm filters to keep them sterile.Each experiment used a control group that received only DMSO at the matching concentration.

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2.3 MTT Assay: Measuring Cell Viability

To find out if betanin, theobromine or a mix of both can kill cancer cells, we used the MTT assay. We plated 10,000 KB cells in each well of 96-well plates and let them attach for 24 hours.

We then changed the medium to fresh medium that included betanin at 1, 10, 25, 50, or 100 µg/mL, theobromine at the same levels, or both together at their calculated inhibitory concentrations (Combo_IC20, IC50, IC70).

Doxorubicin (25 µg/mL) serves as the positive control since it is a well-known cancer treatment. Untreated cells acted as the negative control. After 24 hours, we added 10 µL of MTT to each well and placed the plates in the incubator for 3 to 4 hours. Live cells turned the MTT into purple crystals. We added DMSO to dissolve these crystals and measured the color using a microplate reader at 570 nm. A deeper color meant more cells survived. Each test was repeated three times for reliable results.

2.4 Assessing ROS Levels in Cells

To check the amount of reactive oxygen species (ROS) in cells, we applied the fluorescent dye DCFH-DA. KB cells were placed in black-bottom 96-well plates and given time to stick. Treatments included:

* Betanin at 1 to 100 µg/mL
* Theobromine at 1 to 100 µg/mL
* Their mixtures at Combo_IC20, IC50, IC70
* Control groups (untreated and positive control when required)

After 24 hours, we washed the cells and added DCFH-DA dye in serum-free medium. Cells stayed with the dye for 30 minutes in the dark. Within the cells, the dye reacts with ROS and glows green. We measured this green light using a plate reader, set at 485 nm for excitation and 530 nm for emission. Higher green light showed higher ROS levels. Each set of tests was run three times.

2.5 Gene Expression Analysis Using qRT-PCR

To study how the treatments change cancer gene activity, we measured the levels of genes involved in cell survival and death. The focus was on the PI3K/AKT/mTOR signaling route and genes that control apoptosis.

KB cells were placed in 6-well plates and exposed to betanin, theobromine, their combinations at different concentrations (Combo_IC20, IC50, IC70), or left untreated as controls. After 24 hours, we collected RNA from each group using TRIzol. We checked the RNA quality and used 1 µg to make cDNA. We then ran qRT-PCR with SYBR Green and specific primers for these genes: PIK3CA, AKT1, mTOR (involved in cell survival), and BAX, BCL2, CASP3 (involved in cell death).

All samples were tested in triplicate. GAPDH served as the control gene. We used the ΔΔCt method to compare gene levels in treated cells to untreated controls. These results showed how the treatments changed gene activity at the molecular level.

3. RESULTS

3.1 MTT Assay: Betanin and Theobromine are more effective at killing cancer cells when used in combination.

According to the MTT experiment, both betanin and theobromine were able to eradicate KB oral cancer cells, and the impact grew with the dosage. Particularly at higher concentrations, such as 50 and 100 µg/mL, betanin was very effective in significantly reducing the number of living cancer cells. Theobromine had a comparable impact, even though it was less powerful than betanin when taken by itself. But the most important discovery came from the combination treatments. In some proportions, theobromine and betanin together induced a stronger effect than any of the substances did alone. In other words, the two substances increased their individual cancer eradication capabilities thus causing a synergistic effect. The results that we have got from Combo_IC70, which was more efficient and at the same time with the drug doxorubicin, were equally good or even better, however, the Combo_IC50 combination led to cell survival of about 50% only.}

In general, these findings imply that using betanin and theobromine in combination may be a more potent and safer approach to kill cancer cells since lower dosages of each chemical are needed to produce the same effects.

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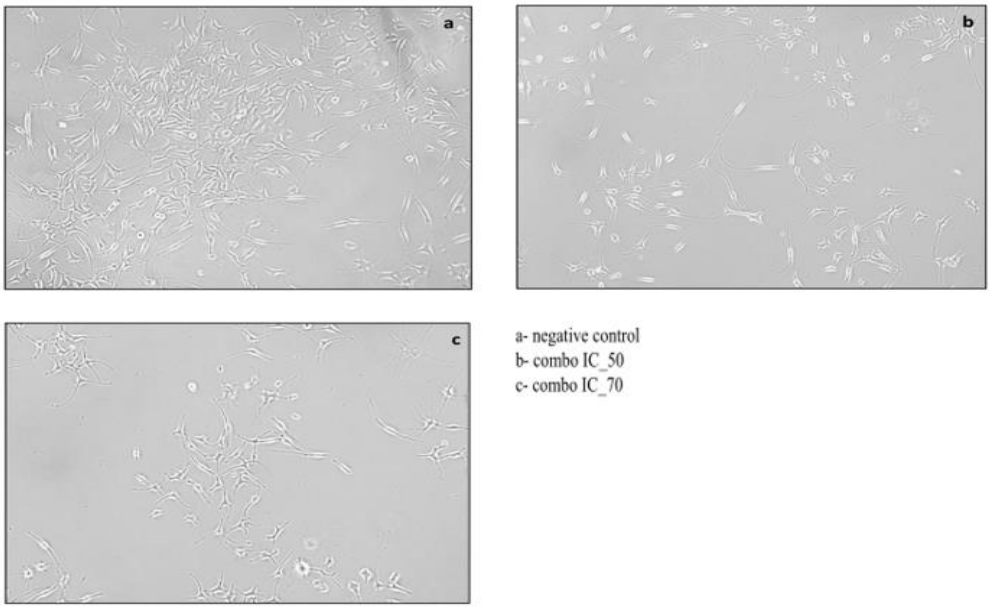

Figure 1: Images Showing How KB Cancer Cells Changed After Treatment

This figure shows microscope images of KB oral cancer cells and how their shape and appearance changed after different treatments:

- (a) Untreated Cells (Negative Control): These cells looked healthy, with their normal long, spindle-like shape. They were well attached to the surface and formed a smooth, even layer.
- (b) Combo_IC50 Treatment: When the cells were treated with a medium dose of the betanin–theobromine combination (IC50), they started to show signs of damage. Some cells shrank, became rounder, and there were fewer cells overall, showing early signs of cell death.
- (c) Combo_IC70 Treatment: At a higher dose (IC70), the changes were much more severe. The cells showed clear signs of late-stage cell death, such as broken membranes (blebbing), detaching from the surface, and breaking apart. This shows the treatment had a strong toxic effect on the cancer cells.

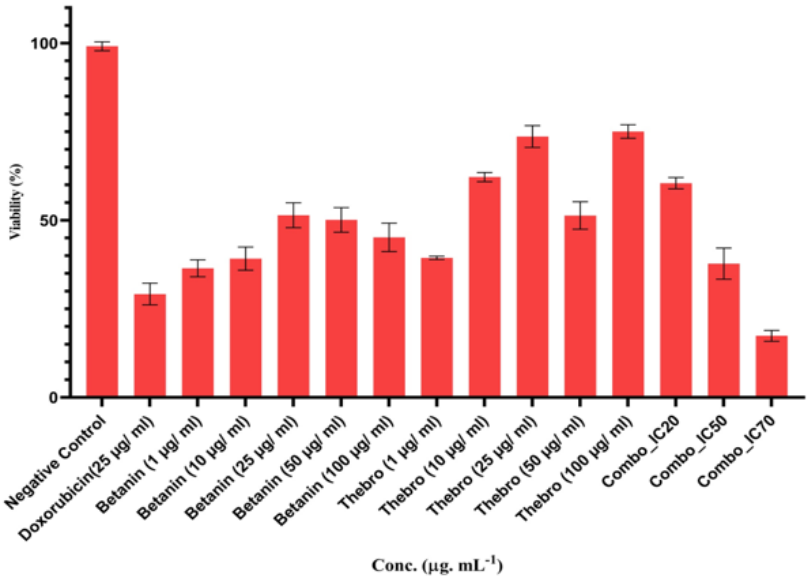

Figure 2: Graph representing the percentage of cell viability in KB cells after 24-hour treatment with various concentrations of betanin (1–100 µg/mL), theobromine (1–100 µg/mL), and their combinations (Combo_IC20, Combo_IC50, Combo_IC70). Doxorubicin (25 µg/mL) was used as the positive control, and untreated cells served as the negative control. Data are expressed as mean ± SD of triplicates. A dose-dependent decrease in cell viability was observed, with combination treatments exhibiting significantly enhanced cytotoxicity compared to individual treatments, indicating synergistic effects.

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3.2 ROS Assay: When combined, bethanin and theobromine increase oxidative stress in cancer cells.
Studies assessed the levels of ROS (reactive oxygen species), which are dangerous molecules that can harm cells and cause cell death, in order to learn how betanin and theobromine affect oxidative stress in cancer cells. The cells are under more stress when there are more ROS present. The health of the cells was demonstrated by the low and normal ROS levels in the untreated cells (negative control). But when doxorubicin, a chemotherapy drug used as a positive control, was given, the ROS levels dramatically rose, proving that the test could accurately detect stress. ROS levels in KB cells treated with betanin stabilized at mid-level doses (25–50 µg/mL) after slightly increasing at lower doses (1–10 µg/mL). It's noteworthy that ROS levels did, in fact, somewhat drop at the maximum dosage (100 µg/mL). This is probably because betanin can lower high levels of oxidative stress because it also possesses antioxidant qualities.

A similar pattern was seen with theobromine. Strong oxidative stress resulted from an increase in ROS at low doses, a slight decrease at mid-levels, and a sharp rise at high doses (50–100 µg/mL). The betanin and theobromine combination treatments produced the most remarkable outcomes. The Combo_IC50 group had the lowest ROS levels of any group, indicating a specific oxidative stress effect potent enough to induce apoptosis, or cell death. Additionally, other combos (Combo_IC20 and Combo_IC70) displayed greater

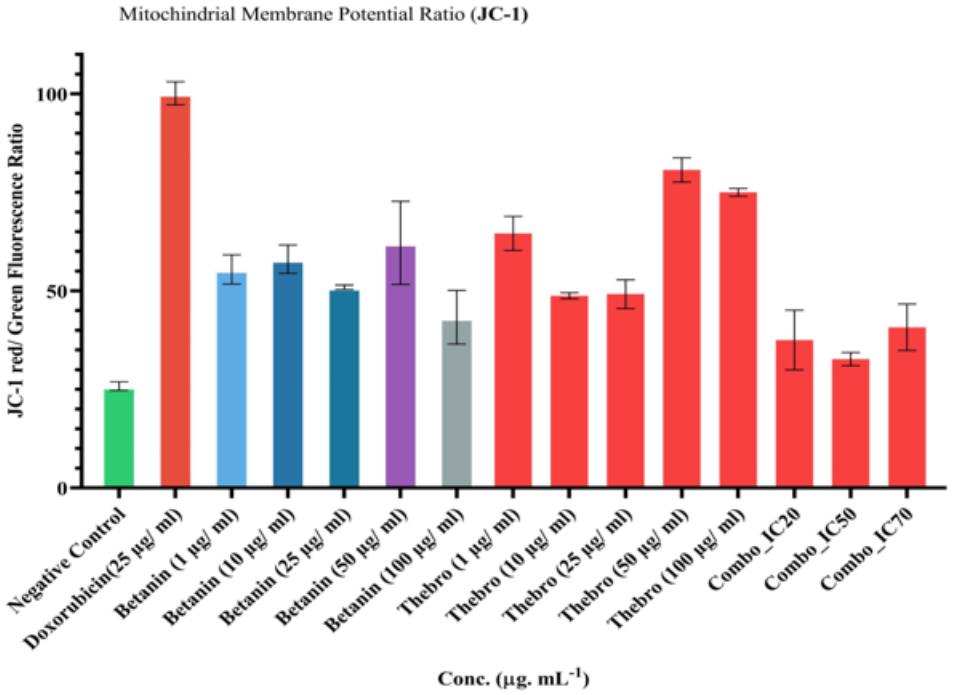

Figure 3: Quantitative analysis of intracellular ROS levels in KB cells treated with varying concentrations of betanin (1–100 µg/mL), theobromine (1–100 µg/mL), and their combinations (Combo_IC20, Combo_IC50, Combo_IC70) for 24 hours. DCFH-DA fluorescence intensity was measured to assess ROS production. Doxorubicin (25 µg/mL) served as the positive control, and untreated cells as the negative control. Data represent mean ROS levels from triplicate experiments, showing a dose-dependent modulation of oxidative stress, with combination treatments inducing significantly elevated ROS compared to individual treatments.

3.3 Gene Expression: Combinatorial Therapy Activates Cell Death and Blocks the Cancer Survival Pathway
Researchers examined the effects of betanin, theobromine, and their combinations on the behavior of specific genes in oral cancer cells (KB cells). By means of the PI3K/AKT/mTOR signaling pathway, these genes support the development and survival of cancer cells. They also examined genes linked to apoptosis, or programmed cell death.

Every gene was operating normally in the untreated cells that made up the control group. The exposure of cancer cells to betanin, especially at higher concentrations (50 and 100 µg/mL), resulted in downregulation of the genes PIK3CA, AKT1, and mTOR, which normally support the growth and survival of cancer cells. While genes that promote cell self-destruction (BAX and CASP3) increased, inhibitors of cell death (BCL2) decreased. .It is possible that betanin blocked the survival signals of the cancer cells, causing them to undergo apoptosis.

Theobromine was slightly less effective than bethanin, but both had similar effects.

As demonstrated by the fact that it raised death-related genes and decreased growth-related genes, it also encouraged the

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death of cancer cells

The most dramatic results came from the combination treatments. When betanin and theobromine were used together — especially at the Combo_IC50 cure — they had the strongest effect

- The survival genes(PIK3CA, AKT1, mTOR) were explosively reduced
- Thepro-death genes(BAX, CASP3) were largely increased
- Theanti-death gene(BCL2) was sprucely dropped

This means the combination was much more important at stopping cancer cell growth and driving cell death than either emulsion alone. These results suggest that using betanin and theobromine together could be a promising new way to fight oral cancer.

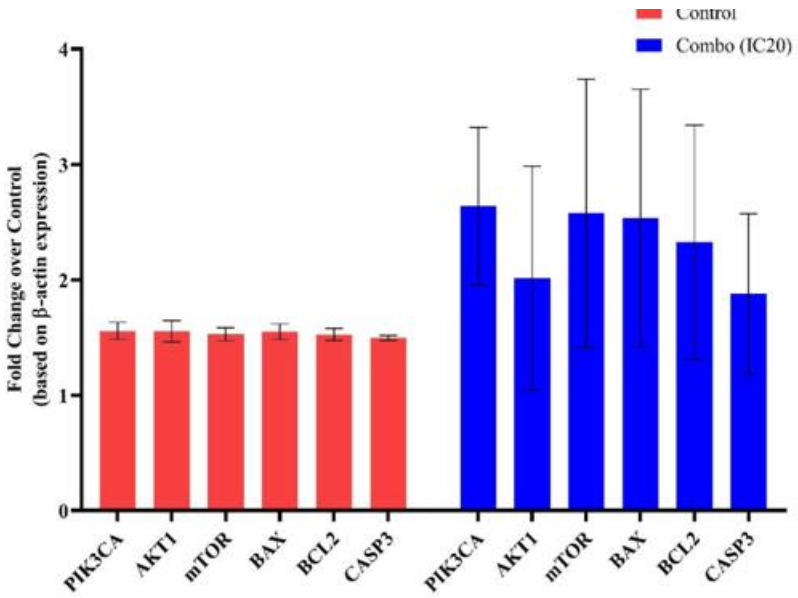

Figure 4: After a 24-hour treatment with betanin (1–100 µg/mL), theobromine (1–100 µg/mL), and combination treatments (Combo_IC20, Combo_IC50, Combo_IC70), the relative mRNA expression levels of PIK3CA, AKT1, mTOR, BAX, BCL2, and CASP3 in KB cells. qRT-PCR was used to quantify gene expression, which was then normalized to GAPDH. Fold change in relation to the untreated control is used to express the data. Synergistic induction of apoptosis via PI3K/AKT/mTOR inhibition was indicated by combination treatments, especially Combo_IC50, which demonstrated significant upregulation of pro-apoptotic genes (BAX, CASP3) and enhanced downregulation of survival pathway genes (PIK3CA, AKT1, mTOR) as well as suppression of anti-apoptotic BCL2.

4. DISCUSSION

4.1 Summary of Results

This study evaluated the anticancer effects of betanin and theobromine, both individually and in combination, on KB oral cancer cells. The results from the MTT assay showed a clear dose-dependent reduction in cell viability, with the combination treatments—especially Combo_IC50 and Combo_IC70—demonstrating significantly higher cytotoxicity compared to single-agent treatments. ROS levels were notably elevated in treated groups, particularly in Combo_IC50, suggesting the induction of oxidative stress. Gene expression analysis revealed downregulation of survival-related genes (PIK3CA, AKT1, mTOR) and upregulation of pro-apoptotic genes (BAX, CASP3), alongside downregulation of anti-apoptotic BCL2, indicating activation of the mitochondrial apoptosis pathway.

4.2 ROS Findings and Comparison

The increase in ROS levels after combination treatment indicates that oxidative stress played a central role in triggering apoptosis in KB cells. Similar findings were reported by (Liu et al. 2023) , who showed that betanin elevated ROS and caused cancer cell death in breast and lung cancer models. Theobromine has also been found to increase oxidative stress in head and neck squamous cell carcinoma (HNSCC) cells (Kim and Kim 2018). On the contrary, some studies highlight betanin's antioxidant role in non-cancerous cells, where it reduces ROS and prevents oxidative damage (Sreekanth et al , 2007). These contrasting results support the dual role of ROS, acting as a protective agent in normal cells but a pro-

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apoptotic trigger in cancer cells when elevated beyond threshold levels.

4.3 Gene Expression Regulation

The qRT-PCR results demonstrated a marked suppression of PI3K/AKT/mTOR signaling components in combination-treated cells, along with significant upregulation of apoptotic markers. Similar outcomes have been observed with phytochemicals like curcumin and sulforaphane, which downregulated mTOR and upregulated BAX/CASP3 in oral and breast cancer cells (Ju et al. 2024; Xie et al. 2021) A study by(Liu et al. 2023) also supports betanin's ability to inhibit AKT/mTOR signaling and promote apoptosis. However, (Gómez-Juaristi et al. 2019) reported that theobromine alone caused minimal changes in gene expression, particularly at low concentrations. Overall, the literature supports the use of natural combinations to enhance gene regulation involved in cancer progression.

4.4 Synergistic effects

The observed community between betanin and theobromine offers a major remedial advantage. The concerted treatment was more effective than either emulsion alone, suggesting commerce between pathways or improvement of each emulsion's effect. This aligns with former exploration where combinations like curcumin and resveratrol showed synergistic apoptosis induction in OSCC cells(Li et al , 2020). Also, quercetin and sulforaphane demonstrated enhanced efficacity through ROS elevation and PI3K inhibition(Yue et al , 2019). Still, studies like (DeNicola et al. 2011)have shown that indecorous combinations, especially those with clashing antioxidant/pro-oxidant places — can lead to enmity. Therefore, the community is largely dependent on cure rates and pathway comity.

4.5 Dose Efficiency and Therapeutic Window

A significant advantage of combination treatment in this study was the effectiveness observed at moderate doses, such as Combo_IC50. Using lower concentrations reduces the risk of toxicity while maintaining anticancer action.(Sharma et al. 2018) reported a similar finding where green tea polyphenols combined with curcumin achieved potent anticancer effects at lower doses. Theobromine's mild nature also supports its use in combination to avoid harsh cytotoxic effects (Kim and Kim 2018). However, some reports, such as (Wang et al. 2016) , noted that higher doses of individual compounds were necessary to observe meaningful gene or protein-level changes in vitro. Even so, most studies agree that synergy can enhance potency and reduce required dosage.

4.6 Role of Natural Compounds

Betanin and theobromine, which come from natural factory sources, may offer a safer choice compared to chemical medicines, especially for treating conditions in their early stages. Numerous studies have shown that factory- grounded composites can fight cancer, reduce inflammation, and cover against cell damage caused by oxidation, all while causing veritably many side goods (Liu et al. 2023; Kim and Kim 2018). In addition, the World Health Organization encourages further exploration on traditional drugs and curatives made from shops for long- term ail.However , natural compounds sometimes face challenges such as poor bioavailability, inconsistent batch quality and limited clinical translation. (Atanasov et al. 2021)Indeed with these issues, strong substantiation shows that further studies on factory- grounded composites as probative treatments are precious and necessary.

4.7 Limitations of the Study

Despite the promising issues of this study, several important limitations should be conceded.

The trials were conducted solely in a controlled laboratory setting, using only one type of oral cancer cell line(KB), which limits the capability to directly apply these findings to real natural systems(Fathima et al. 2020). To strengthen the substantiation, unborn exploration should include studies in beast models and tests using a variety of oral cancer cell lines. Another crucial limitation is the absence of studies on normal oral epithelial cells, leaving a query about whether the treatment specifically targets cancer cells or also affects healthy towels. The duration of the treatment period was fairly brief, limited to 24 hours, and extended exposure might lead to different issues related to cell death. Also, while changes in gene expression were observed, vindicating these findings at the protein position through ways similar as Western blotting or ELISA would give a more accurate understanding of how the treatment functions in practice.

4.8 Future Scope and Applications

Looking ahead, further exploration should concentrate on assessing these findings in beast models to better understand how the treatment behaves within a complete natural system and to assess any implicit side effects. Following this, clinical trials would be necessary to determine safe lozenge situations and overall safety for mortal use. It would also be salutary to test the treatment on normal oral cells to confirm whether its goods are picky for cancerous cells. The development of nano- reprised delivery systems could enhance the stability and immersion of the composites within the body. Also, exploring specific molecular pathways and combining these composites with conventional chemotherapy medicines may enhance their effectiveness. With continued disquisition, betanin and theobromine may prove to be a low- toxin, effective reciprocal treatment for oral cancer.

5. CONCLUSION

In simple terms, betanin and theobromine kill KB oral cancer cells more effectively when combined than when used separately. Together, they cause the cancer cells to experience more oxidative stress, inhibit important growth pathways (PI3K, AKT, and mTOR), and initiate programmed cell death. . Most importantly, these goods do at lower doses, making the combination potentially safer and further effective for unborn cancer treatments.

According to these results, plant-based substances may prove beneficial as novel or supplemental therapies for oral cancer. To help bring this natural combination closer to being used in actual cancer treatment, more research is required, including animal and human trials.

6. AUTHOR CONTRIBUTIONS

Aishwarya Kapoor - contributed in designing the study, execution of the project, statistical analysis, manuscript drafting.
Dr.K.Meenakshi Sundaram - contributed in designing the study, execution of the project, statistical analysis, manuscript drafting.
Mrs.S.Sangeetha - contributed in study design, guiding the research work, manuscript correction and publication.

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8. CONFLICT OF INTEREST

The authors hereby declare that there is no conflict of interest in this study.

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