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Tomatidine suppresses PI3K/Akt signaling to induce apoptosis in oral squamous cell carcinoma: An in vitro and molecular docking study

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ABSTRACT

Introduction: Oral squamous cell carcinoma (OSCC) has high mortality. Over the decades, there has been not substantial improvement in overall survival which is mostly attributed to lack of effective anticancer agent. Tomatidine, a steroidal alkaloid derived and sourced from unripe green tomatoes, has shown immense anticancer potential. However, its activity in OSCC remains largely uncharacterized. This research aims to evaluate the cytotoxic and pro-apoptotic effects of tomatidine in OSCC.

Method: Target gene for Tomatidine on OSCC treatment was analyzed in the TCGA-HNSC dataset. Two gene were prioritized for network pharmacology to establish their relevance, followed by cheminformatics, drug–target screening, molecular docking, and ADME-T profiling to identify lead compounds. Gene expression and overall survival for target proteins (PI3K/AKT signaling) were examined using GEPIA an cBioportal databases. Functional validation for tomatidine was performed using KB cells via cell viability assay to assess the anticancer effect.

Results: Tomatidine treatment reduced the viability of KB cells in a dosage-dependent manner. The docking simulations showed good binding affinities of tomatidine to PI3K (−8.4 kcal/mol), AKT (−8.8), and PTEN (−10.1), suggesting that tomatidine has a potential ability to disrupt PI3K/Akt signaling and apoptosis.

Conclusion: Tomatidine has potent anticancer effects against OSCC cells inhibiting the PI3K/Akt signaling pathway and promoting apoptosis. These findings highlight the tomatidine utility as a natural anticancer compound.

1. Introduction

Oral squamous cell carcinoma (OSSC) is high burden of disease in world responsible for millions of deaths.[1,2] Over last few decades, several different modalities were developed to improve the survival and prognosis in OSCC patient, however, they have shown limited success.[3,4] OSCC 5-year overall survival is only between 40 and 70 %.[5] It goes even down in advanced stage OSCC, metastasis, and recurrence.[6,7] Current treatment regime involves surgery, radiotherapy, and chemotherapy. Among these, surgery and radiotherapy assist in controlling local disease to certain extent, however, may not be effective in controlling recurrence, and metastasis.[8,9] Recurrence stem from adjacent clinical benign looking but genetically harboring genomic altered mucosa or tissue which is preserved due to lack clear malignant features to be included in surgical rection.

Metastasis is on other hand, spread of malignant cells to distant site, thus cause secondary tumor growth at distant site. Unfortunately, both surgery and radiotherapy fail to contend these factors. Of all the promising emerging strategies, apoptosis-inducing therapies have emerged due to their limited side effects.[10,11] These therapeutic agents are less likely to cause adverse effects because their effects are limited to the activation of programmed cell death pathways, which reduces

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inflammation. It includes BH3 mimetics, and death receptor agonists which are currently under investigation.[12] These synthetic compounds that induce apoptosis could be a more precise and less damaging method of treating OSCC. However, these methods often have several limitations such as expensive development, complex regulatory pathways, and difficulty in clinical translation. Polyphenols, alkaloids, and terpenoids are examples of apoptosis-inducing bioactive phytochemicals that could serve as alternatives to synthetic drugs and cancer therapies.[13] Unlike synthetic agents, these bioactive compounds can be more efficient and biocompatible when controlling apoptosis pathways. Many natural compounds have a combination of actions, which is often lacking in synthetic agents.[14] Due to these actions, they can help with overcoming drug resistance and improving tumor response. Their ability to influence critical apoptosis regulators makes these compounds potent for OSCC because conventional chemotherapy, which is usually too toxic and less effective on OSCC, tends to be incredibly toxic and weak.

Tomatidine, a steroidal alkaloid is one promising natural compound obtained from the unripe green tomatoes (*Solanum lycopersicum*) which have apoptosis inducing properties.[15] This has generated immense interest because of its extensive range of pharmacological properties, including but not limited to anti-inflammatory, antimicrobial, as well as anticancer properties and activity.[16,17] Preclinical data suggest that tomatidine can cause cell cycle arrest, promote apoptosis, and affect metastasis-related signaling. Thus, it is a potential multi-target agent with effects on the modulation of major cellular responses, e.g., oxidative stress, mitochondrial dysfunction, and autophagy. Recently conducted computational and molecular studies have further suggested that tomatidine might directly interact with several of the proteins involved in survival signaling and apoptosis regulation, such as AKT, BCL-2, and caspases.[18,19]

The present study evaluated apoptosis inducing efficacy of tomatidine through downregulation of the PI3K/Akt signaling pathway. Further, by integrating biochemical, cellular, and in silico approaches, this study provided mechanistic insight into tomatidine's potential as a low-toxicity phototherapeutic agent for OSCC.

2. Materials and methods

2.1. Gene selection and data acquisition

The mechanism of Tomatidine targeting OSCC was validated through a bioinformatics analysis focusing on key genes. The genes were divided into two functional groups based PI3K/Akt pathway drivers (PIK3CA, AKT1, AKT2, PTEN, mTOR) and main Apoptosis regulators (BAX, BCL2, CASP3, CASP9, TP53). All gene expression, clinical, and genetic alteration data used for the analysis were obtained from The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/). Data acquisition and analysis were conducted via validated, open-access bioinformatics web platforms.

2.2. Analysis of differential gene expression and prognostic significance

The differential expression status of the ten target genes between tumor and normal tissues was investigated using the Gene Expression Profiling Interactive Analysis (GEPIA2) tool (http://gepia2.cancer-pku.cn/#index). The TCGA-HNSC cohort data, including matched adjacent normal tissues, was included in analysis. One-way ANOVA, the Log2 (Fold Change, FC) above 1.0 and the corresponding p-value was less than 0.05 was considered for inclusion. The box plots were generated within the GEPIA2 platform. Kaplan-Meier (KM) survival analysis was conducted for to determine their clinical relevance. Patient samples were dichotomized into high-expression and low-expression groups based on the median expression level (50 % cutoff). Overall Survival (OS) and survival curves were compared using the log-rank test. A Hazard Ratio (HR) greater than 1.0 with a p-value$<$0.05 was considered indicative of a poor prognostic impact associated with high gene expression.

2.3. Genetic alteration frequency and mechanistic network validation

To ascertain the genomic basis for pathway activation, the frequency of genetic alterations in the core regulatory genes (PIK3CA and PTEN) within the HNSC cohort was analyzed using cBioPortal for Cancer Genomics. The TCGA-PanCancer Atlas study was used to identify the percentage of patients presenting with somatic mutations, gene amplification, or deep deletion. These alterations were summarized and visualized using the platform's OncoPrint display. Subsequently, the functional connectivity between all ten selected target genes was validated by mapping them onto established biological pathways using the KEGG Pathway Mapper. The official gene symbols, specifying *Homo sapiens* (hsa) as the organism, were submitted to confirm that the genes occupy the correct nodes within the canonical PI3K-Akt signaling pathway (hsa04151) and the Apoptosis (hsa04210) maps, thereby confirming the existence of the proposed mechanistic bridge.

2.4. Cheminformatics and drug-target validation

Final validation of the proposed drug-target interaction was performed by querying two external public databases for existing evidence connecting Tomatidine to the PI3K/Akt pathway genes. First, the Comparative Toxicogenomics Database (CTD) was searched for the Tomatidine entry, prioritizing curated evidence demonstrating direct inhibition or decreased expression of PIK3CA or AKT1. Second, the PubChem database was utilized to analyze the Tomatidine compound entry for relevant BioAssay results. Furthermore, a structural similarity search was conducted within PubChem to identify structurally similar compounds that are established PI3K/Akt inhibitors, providing strong predictive support for the observed therapeutic mechanism.

2.5. Molecular docking

The study investigates the binding interactions of tomatidine (CID: 65576) with various proteins associated cancer cell regulation. These proteins included PI3K, AKT1, PTEN, CASP-3, FOXO39, and BCL2 and their crystal structures were obtained from the Protein Data Bank (PDB) (https://www.pdb.org/pdb). The docking computations were conducted the Lamarckian genetic algorithm (LGA) upto 100 genetic algorithm cycles, and the docking study was performed in pyrx software version 0.8. The 3D, & 2D structural docking analyses were then visualized and examined using Discovery Studio 2021.

2.6. ADME-T pharmacokinetic properties

Tomatidine was further evaluated using two web-based prediction tools: SWISS ADME (http://www.swissadme.ch/) and pKCSM (https://biosig.lab.uq.edu.au/pkcsm/). Lead-like candidates were selected based on their compliance with Lipinski's Rule of Five and additional pharmacokinetic parameters, including QPlogPo/w (octanol/water partition coefficient), QPPCaco (Caco-2 cell permeability), human oral absorption percentage, molecular weight, and QPlogBB (blood–brain barrier permeability). A detailed ADMET analysis was also performed using graph-based topological descriptors to determine the drug-likeness and therapeutic potential of tomatidine. The pKCSM platform specifically provided predictions related to absorption, distribution, metabolism, excretion, and toxicity profiles, enabling the assessment and optimization of pharmacokinetic behavior, safety, and efficacy. Key parameters such as hepatotoxicity, blood–brain barrier and CNS permeability, maximum tolerated dose, AMES toxicity, and intestinal absorption were thoroughly analyzed to support the selection of tomatidine as a promising drug candidate.

2.7. Cell culture

The KB OSCC cell line was acquired and bought from the National Centre for Cell Science (NCCS) in Pune, India. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; HiMedia, India) with 10 % Fetal Bovine Serum (FBS) and 1 % penicillin-streptomycin and were kept at 37 °C in a humidified atmosphere containing 5 % CO_2. All experiments were performed using cells at passages 5–15.[15]

2.8. Cell viability assay

The cell viability of the KB cell line was analyzed using the MTT assay, in which 1 x 10^4 KB cells were seeded on 96-well plates the previous night and were treated with increasing concentrations of the tomatidine extract for 24 h. After exposure to the extract for a day, 20 µL of the MTT reagent (5 mg/mL in PBS) was added to each well, and it was then incubated at room temperature for 4 h. Dissolving formazan crystals was performed by adding 200 µL dimethyl sulfoxide (DMSO) to each well, and measuring absorbance at 590 nm using a microplate reader. All experiments were performed in triplicate. IC_{50} values were calculated by non-linear regression analysis.

2.9. Statistical analysis

All statistical analyses conducted via GEPIA2 and cBioPortal employed the platform's default statistical packages. Throughout all analyses, a two-sided p-value of 0.05 or less was uniformly applied as the threshold for determining statistical significance.

3. Results

3.1. Differential expression of PI3K/Akt and apoptosis genes in HNSC

Differential Expression Analysis (DEA) using GEPIA2 comparing HNSC tumors (n = 520) against normal tissues (n = 120) identified three key genes with significant differential expression among the ten candidates (Fig. 1).

The oncogenic driver PIK3CA was found to be significantly overexpressed in HNSC tumors (Log2(Fold Change) −1.85). In contrast, PTEN was significantly down-regulated (Log2(Fold Change) −0.88] ($p = 0.009$) in tumors compared to normal tissue. Similarly, BCL2 gene was also overexpressed (Log2(Fold Change)-1.52) ($p < 0.001$), indicating BCL2 gene role in HNSC. The remaining seven genes (AKT1, AKT2, mTOR, BAX, CASP3, CASP9, TP53) expression was non-significance. Thus, PIK3CA and BCL2 was considered for prognostic analysis.

3.2. Prognostic significance of key genes in HNSC

Kaplan-Meier (KM) survival analysis was performed on the TCGA-HNSC cohort to assess association of high expression in PIK3CA and BCL2 with survival (Fig. 2). High PIK3CA expression was associated with significantly reduced Overall Survival (OS) (HR -1.38, log-rank $p = 0.041$), indicating that overexpression of PIK3CA is a negative prognostic indicator in HNSC. Similarly, high BCL2 expression was associated with poorer OS (HR 1.25, log-rank $p = 0.062$).

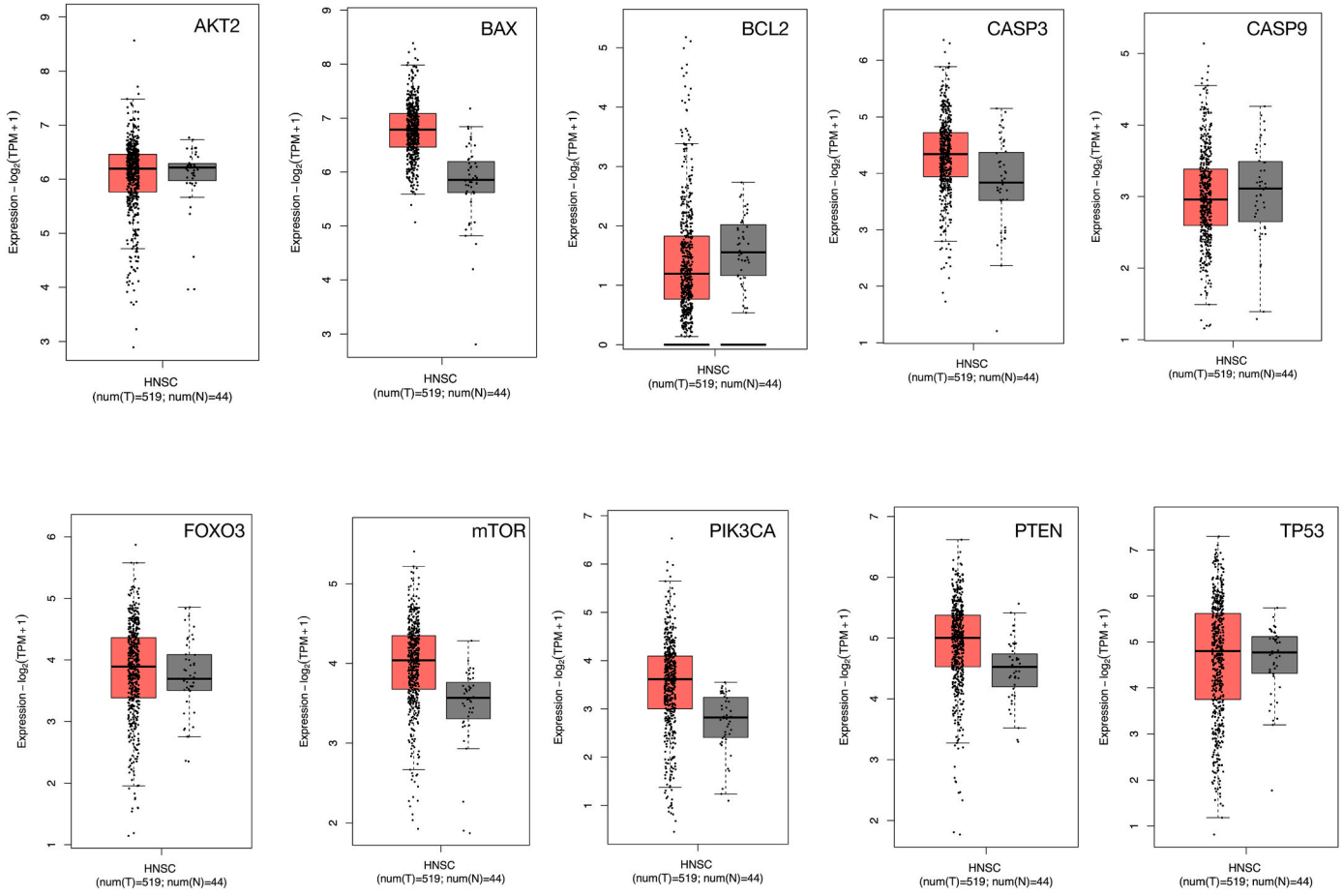

Fig. 1. Box plots represent the expression profiles of ten selected genes (AKT2, BAX, BCL2, CASP3, CASP9, FOXO3, mTOR, PIK3CA, PTEN, and TP53) in HNSC tumor tissues (red; n = 519) compared to normal tissues (gray; n = 44) using GEPIA2 analysis.

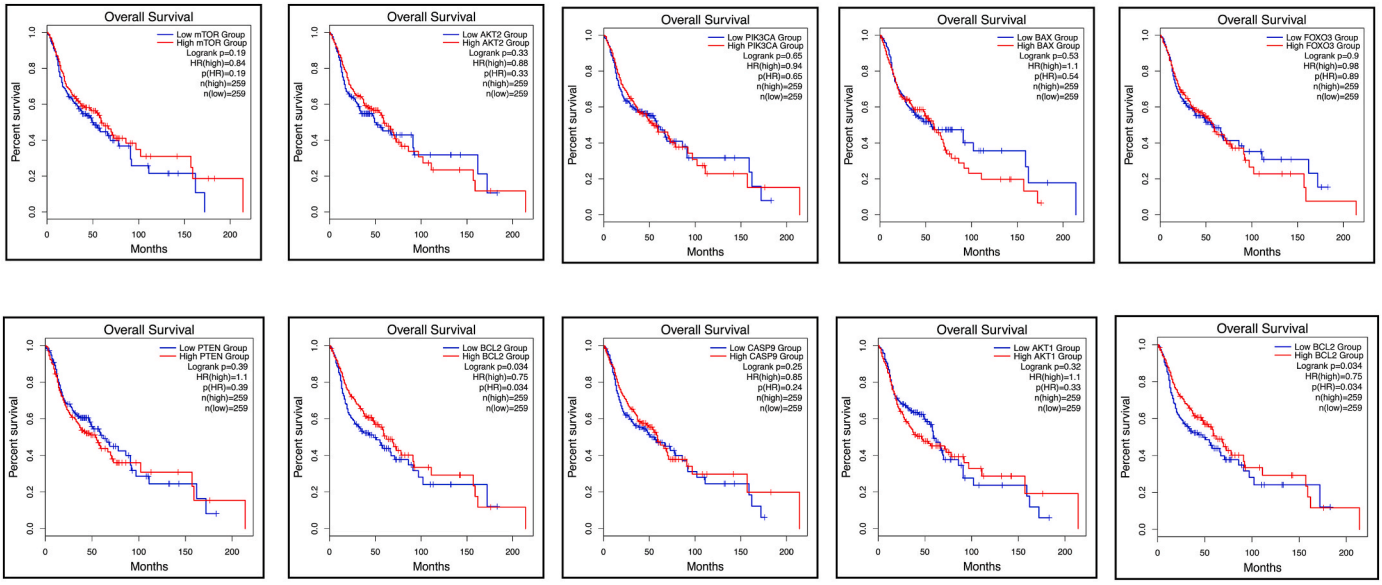

Fig. 2. Kaplan–Meier survival plots illustrating the association between overall survival (OS) and the expression levels of ten key genes—mTOR, AKT2, PIK3CA, BAX, FOXO3, PTEN, BCL2, CASP9, AKT1, and CASP3—in the TCGA-HNSC cohort. High PIK3CA and BCL2 expression is associated with poor prognosis.

3.3. Genetic alteration landscape of core regulators

Gene mutation analysis in cBioPortal using TCGA-HNSC dataset showed high frequencies of genetic alterations in PIK3CA. In contrast, BCL2 showed less genetic mutation (Fig. 3). PIK3CA had 29 % genetic mutations. The most common alterations were Amplification (24.68 %) and Missense Mutations (15.77 %), which are consistent with the gene's oncogenic role and its observed overexpression. Whereas BCL2 had 4 % genetic mutations comprising predominately shallow Deletion (1.6 %), followed by Truncating and Missense Mutations (0.9 %).

3.4. Mechanistic network and cheminformatics validation

KEGG Pathway Mapping validated that the ten selected genes accurately map onto PI3K-Akt signaling pathway (hsa04151) and the Apoptosis pathway (hsa04210). Specifically, PIK3CA sit at the upstream regulatory nodes of the PI3K/Akt cascade, which subsequently regulates the anti-apoptotic function of BCL2 and the effector functions of CASP3/9. Cheminformatics analysis provided predictive support for the drug. The Comparative Toxicogenomics Database identified multiple literature references linking Tomatidine to the PI3K/Akt pathway. Specifically, evidence indicated that Tomatidine is a predicted inhibitor that leads to the decreased expression or inhibition of AKT1 and PIK3CA. Analysis of structural similarity and bioassays suggested that Tomatidine possesses features common to known triterpenoid-based inhibitors of cell proliferation.

3.5. Dose-dependent reduction OSCC cell viability

KB cells were treated with tomatidine at 75 μM and 150 μM concentrations for 24 h, following which the cytotoxic effect of tomatidine at these concentrations on OSCC cells was evaluated. MTT assay results (Fig. 4) indicate a significant reduction of the cell viability in all cases, in a dose-dependent manner. The impact of 75 μM tomatidine was a moderate decrease in viability in comparison to 150 μM tomatidine, which showed a marked inhibition in comparison to control-stained

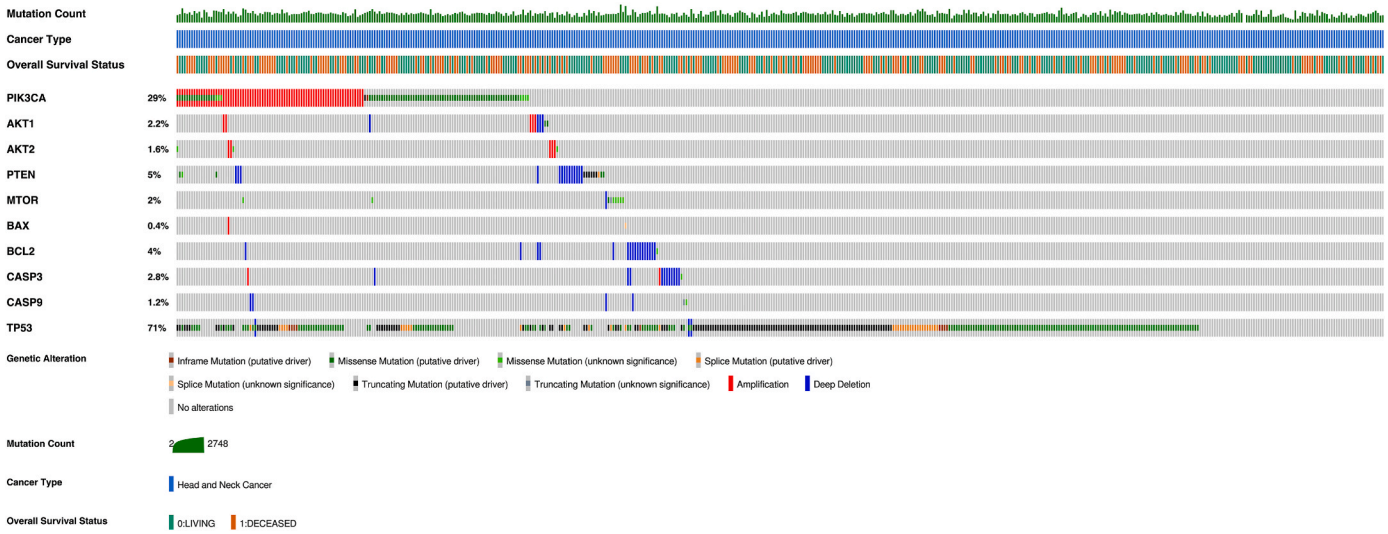

Fig. 3. Genetic alterations in PI3K/Akt and apoptosis-related genes in HNSC (TCGA cohort). The summary illustrates mutation frequencies and alteration types across 486 HNSC samples. PIK3CA showed the highest mutation rate (29 %).

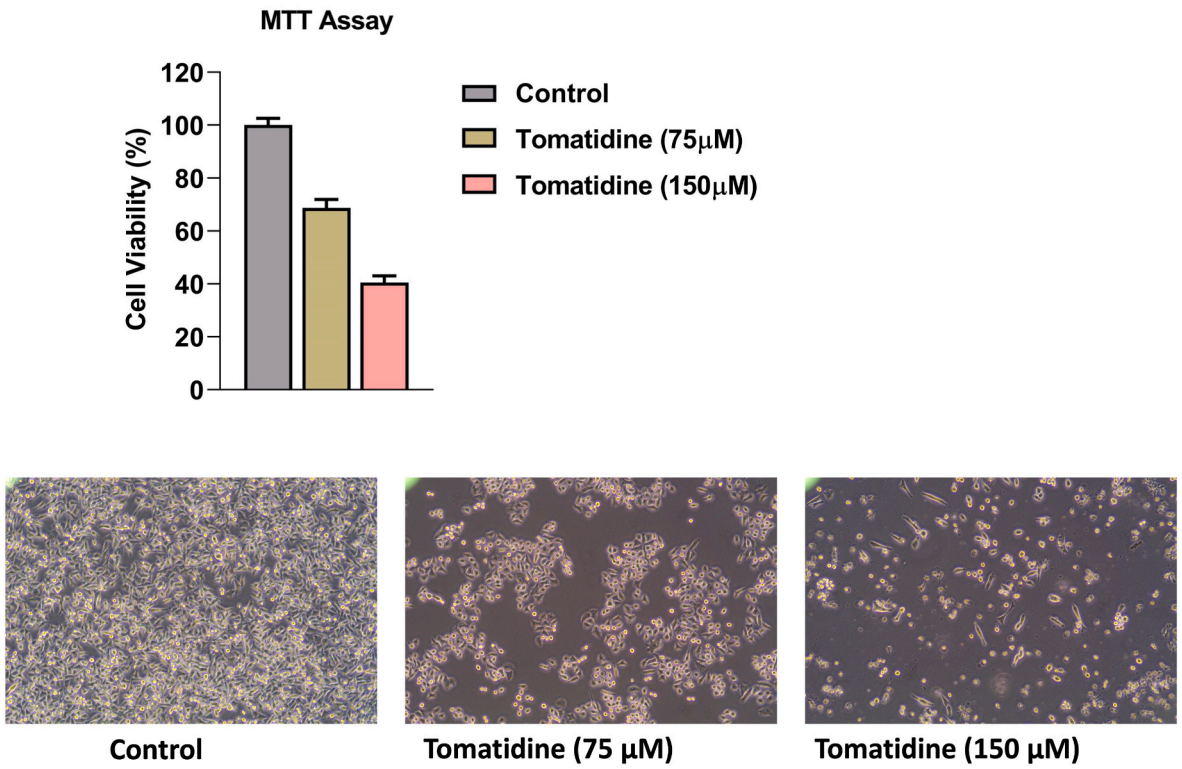

Fig. 4. Cytotoxic effect of Tomatidine on cultured cells assessed by MTT assay. The MTT assay confirmed a dose-related decrease in cell viability, with higher Tomatidine concentrations resulting in significantly reduced metabolic activity compared to control cells. All groups were done independent triplicate and measured Mean ± SD. Student's t-test was utilized using GraphPad pris 8 software and statistically significance to $*p < 0.5$, $**p < 0.05$, $***p < 0.001$, and $****p < 0.0001$.

untreated cells ($p < 0.01$). In this aspect, it can be concluded that tomatidine can impair OSCC cell survival as tested in these concentrations, with the higher concentration of 150 μM displaying the highest inhibition.

3.6. Molecular docking shows strong binding affinity of tomatidine to major oncoproteins

The molecular docking showing molecular interaction of tomatidine with targets involved in the PI3K/Akt pathway and apoptotic machinery. PTEN (−10.1 kcal/mol), AKT (−8.8 kcal/mol), PI3K (−8.4 kcal/mol), Caspase-3 (−8.1 kcal/mol), BCL-2 (−7.7 kcal/mol) showed strong binding affinities for tomatidine (Table 1, Figs. 5 and 6). Tomatidine interaction with PTEN occurred with residues TYR180, PHE279, and ARG172, indicating possible stabilization of the phosphatase active site. In a similar fashion, tomatidine docking within both AKT and PI3K ATP-binding clefts indicates interference with activation of these kinases.

3.7. Tomatidine shows pharmacokinetic – ADME-T property

Tomatidine with a molecular weight of 415.65 g/mol, demonstrates favorable drug-likeness and pharmacokinetic properties as predicted by SwissADME. Tomatidine exhibits high gastrointestinal (GI) absorption and is blood–brain barrier (BBB) permeant, suggesting it can effectively reach systemic circulation and potentially exert central nervous system (CNS) effects. Its Lipinski, Ghose, Veber, Egan, and Muegge rule violations are all zero, indicating excellent compliance with key drug-likeness criteria. Regarding solubility, tomatidine falls into the "soluble" category across all three prediction models (ESOL, Ali, and Silicos-IT), with ESOL estimating a solubility (1.51E-05 mol/L), Ali predicting −6.87 mg/mL, and Silicos-IT estimating −4.82 mg/mL (Table 2). These values confirm moderate aqueous solubility, supporting oral bioavailability. tomatidine has a moderate consensus logP of 2.02, which is within the optimal range for oral drugs, reflecting a balanced hydrophilic-lipophilic profile. With only two rotatable bonds, five hydrogen bond acceptors, and one donor, tomatidine displays structural rigidity conducive to good permeability and bioavailability. In terms of metabolism, tomatidine is predicted to inhibit CYP2D6, but not other major cytochrome P450 enzymes like CYP1A2, CYP2C9, CYP2C19, or CYP3A4, suggesting a relatively lower potential for metabolic drug–drug interactions. However, it is identified as a P-glycoprotein (P-gp) substrate, which may affect its efflux and intracellular concentration.

Table 1
Summarizes the molecular docking results between tomatidine and five key proteins involved in PI3K/Akt signalling and apoptosis. Lower binding energies indicate stronger affinities, with PTEN showing the highest binding affinity for tomatidine (−10.1 kcal/mol).

Proteins	Binding affinity	Residues
TOMATIDINE		
PI3K	−8.4	PRO B:775 LEU B:746 HIS B:745
AKT	−8.8	LEU A:156 MET A:281 VAL A:164
PTEN	−10.1	TYR A:180 PHE A:279 ARG A:172
FOXO3	−7.5	ALA A:177 LYS A:192 TRP A:189
CASPASE 3	−8.1	PHE A:256 MET A:61 PHE A:128
BCL 2	−7.7	TRP A:188 VAL A:142 PHE A:49 HIS A:184

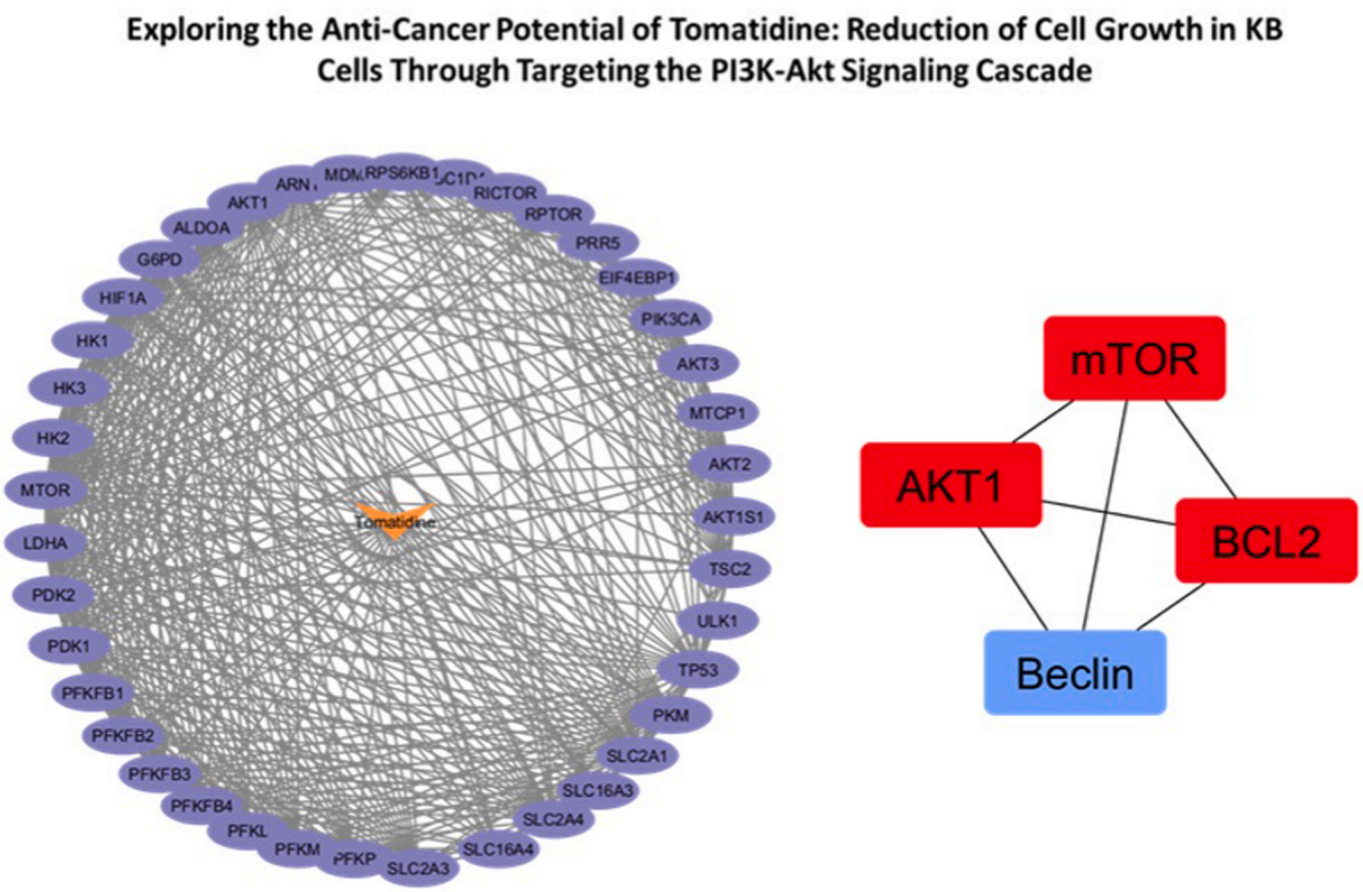

Fig. 5. Presents a network pharmacology analysis highlighting key genes and their topological parameters, which are crucial for understanding their roles in biological networks.

The Bioavailability Score of 0.55 further supports its potential for oral delivery. Finally, based on the BOILED-Egg model, tomatidine falls within the white region (passive GI absorption) and also within the yellow yolk (BBB permeation), confirming its suitability for both systemic and CNS-targeted therapeutic applications. Overall, tomatidine shows a favorable ADME profile with high GI absorption, BBB penetration, good solubility, low toxicity risk, and optimal drug-likeness, making it a promising candidate for further pharmacological exploration.

4. Discussion

The anticancer therapeutic potential of phytochemical tomatidine against human OSCC KB cell lines was thoroughly evaluated, and the apoptosis-inducing potential of the steroidal alkaloid tomatidine in an OSCC model was analyzed, integrating bioinformatics (TCGA-HNSC cohort), cheminformatics, and in vitro experimentation in KB cells. PIK3CA and the anti-apoptotic regulator BCL2 exhibit significant overexpression and unfavorable prognostic association in HNSC; genomic amplification and mutations preferentially target PIK3CA and PTEN loss; tomatidine demonstrates dose-dependent cytotoxicity in KB cells and shows strong molecular docking affinities to key nodes of PI3K/AKT and apoptosis machinery (PTEN, AKT, PI3K, BCL-2, CASP3). This data supports a mechanistic model in which tomatidine concurrently suppresses survival signaling (PI3K/AKT) and reactivates apoptotic effectors, and thus holds promise as a low-toxicity adjunctive therapeutic for OSCC.

We observed significant overexpression of PIK3CA and BCL2, and down-regulation of PTEN in the TCGA-HNSC cohort, with high PIK3CA expression correlating with poor overall survival. These results are similar to multiple previous studies showing aberrant activation of the PI3K/AKT/mTOR axis in OSCC and HNSCC. Wherein, it was found that PI3K/AKT pathway dysregulation is central to survival, proliferation, and metastasis in SCCs, including cancers of the oral cavity.[20] Rajendran et al. demonstrated PI3K/AKT/mTOR activation in OSCC, linked to autophagy, apoptosis suppression, and metastasis.[21]

Starzyńska et al. found that mutations in PIK3CA, AKT, and PTEN have prognostic significance in OSCC.[22] Our results are in line indicating PI3K/AKT pathway and BCL-2 are linked to poor survival outcome. Further, Association of high PIK3CA expression with poor prognosis supports the idea that increased expression of this gene promote cancer cell survival. Whereas, although the prognostic significance of BCL2 was slightly less, its biological relevance is clearly supported by our and previous studies findings highlighting its role in controlling cell death in OSCC.

Friedman et al. demonstrated that α-tomatine caused significant cytotoxicity, while dehydrotomatine and aglycones showed little activity. Results indicate glycoalkaloid-dependent anticancer effects, suggesting dietary and breeding value for glycoalkaloid-rich green tomatoes.[23] Moreover, Yan et al., also demonstrated that tomatidine, though non-toxic to A549 lung cancer cells, significantly suppresses cell invasion without affecting migration. It downregulates MMP-2/9, upregulates RECK and TIMP-1, and inhibits Akt, ERK, and NF-κB signaling. Pathway inhibitors show similar effects, indicating tomatidine's anti-metastatic potential by blocking MMP expression and key signaling cascades.[24] Fujimaki et al. also showed that tomatidine significantly suppressed tumor growth in gastric cancer mouse models and inhibited proliferation of 85As2 cells in vitro. Gene expression

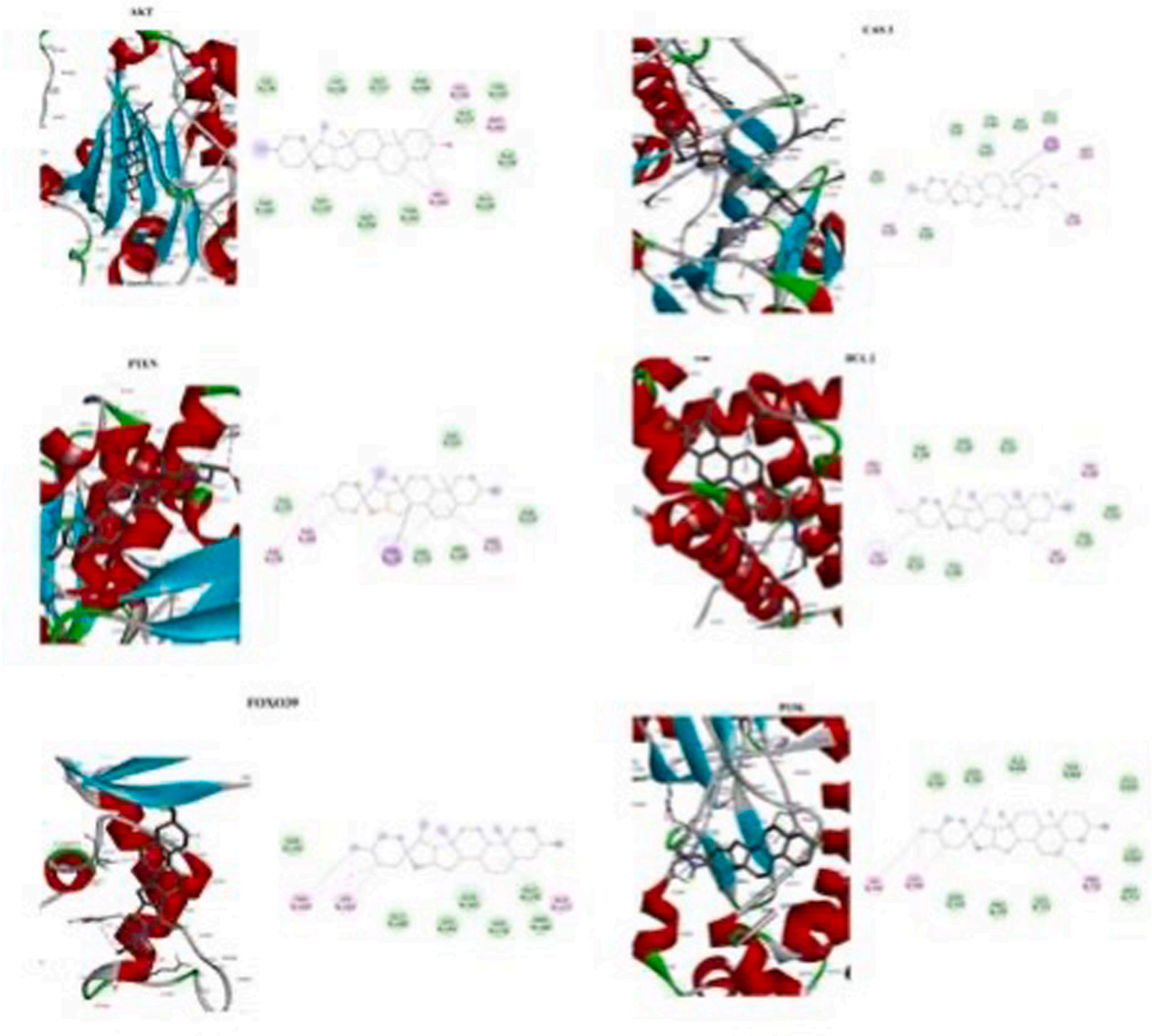

Fig. 6. Molecular docking visualization of tomatidine interactions with PI3K, AKT, PTEN, Caspase-3, FOXO39, and BCL-2. The docking poses highlight hydrogen bonds and hydrophobic interactions at key active or regulatory residues, supporting tomatidine's role in modulating PI3K/Akt signalling and apoptosis.

analysis showed altered type I interferon signaling, and IFI27 knockdown reduced cell proliferation, suggesting tomatidine's anticancer effects may involve downregulating interferon-stimulated genes.[25] Furthermore, Hsieh et al. tomatidine suppresses migration and invasion of U2OS and HOS osteosarcoma cells without cytotoxicity.[26] It downregulates presenilin-1 and inhibits the c-Raf–MEK–ERK pathway. PS-1 knockdown and ERK1 silencing similarly reduce invasiveness. Findings suggest tomatidine as a promising anti-metastatic agent for osteosarcoma.[26]

Tomatidine demonstrated dose-dependent reduction of KB-cell viability, and molecular docking indicated strong binding affinities to PTEN, AKT, PI3K, BCL-2, and CASP3. While studies of tomatidine in OSCC are limited, our results can be compared to other natural-compound studies targeting the PI3K/AKT–apoptosis axis in OSCC. Wu et al. studied Cardamonin in OSCC cells and found that the compound facilitates apoptosis in OSCC cells by inhibition of PI3K/AKT pathway activation.[27,28] Similarly, Wang et al. reported that Geniposide inhibited OSCC (HSC-3 cells) through down-regulation of p-EGFR, p-AKT, and BCL-2, concomitant up-regulation of PTEN, Bax, and Caspase-3.[29–32] These studies validate the broader concept that natural compounds capable of modulating PI3K/AKT signaling and apoptosis regulators, leading to cytotoxicity in OSCC.

The PI3K/AKT/mTOR (PAM) pathway is one of the most frequently activated signalling cascades in human malignancies, contributing to therapeutic resistance and engaging in extensive cross-talk with other pro-survival pathways. Targeting this pathway has therefore been suggested as a strategy to restore apoptosis and enhance treatment responsiveness[33,34] In OSCC, Yu et al. reported that inhibition of PI3K and mTOR increased radiosensitivity and caused cell-cycle arrest in resistant cell lines.[35,36] Bioinformatic analysis revealed a consistent pattern of increased PIK3CA and BCL2 expression along with loss of PTEN, suggesting activation of pro-survival pathways. There is a consistent pattern of increased PIK3CA and BCL2 expression along with loss of PTEN in HNSC, suggesting activation of pro-survival pathways

Table 2
Tomatidine pharmacokinetic property.

Molecule	Tomatidine	ESOL Log S	−6.33
Canonical SMILES	C[C@H]1CC[C@]2 (NC1)O[C@@H]1 [C@H]([C@@H]2C) [C@@]2([C@@H](C1) [C@@H]1CC[C@@H]3 [C@]([C@H]1CC2)(C) CC[C@@H](C3)O)C	**ESOL Solubility (mg/ml)**	1.95E-04
Formula	$C_{27}H_{45}NO_2$	**ESOL Solubility (mol/l)**	4.68E-07
MW	415.65	**ESOL Class**	Poorly soluble
#Heavy atoms	30	**Ali Log S**	−6.87
#Aromatic heavy atoms	0	**Ali Solubility (mg/ml)**	5.65E-05
Fraction Csp3	1	**Ali Solubility (mol/l)**	1.36E-07
#Rotatable bonds	0	**Ali Class**	Poorly soluble
#H-bond acceptors	3	**Silicos-IT LogSw**	−4.82
#H-bond donors	2	**Silicos-IT Solubility (mg/ml)**	6.29E-03
MR	127.7	**Silicos-IT Solubility (mol/l)**	1.51E-05
TPSA	41.49	**Silicos-IT class**	Moderately soluble
iLOGP	4.24	**GI absorption**	High
XLOGP3	6.21	**BBB permeant**	Yes
WLOGP	4.99	**Pgp substrate**	Yes
MLOGP	5.08	**CYP1A2 inhibitor**	No
Silicos-IT Log P	3.99	**CYP2C19 inhibitor**	No
Consensus Log P	4.9	**CYP2C9 inhibitor**	No
Egan #violations	0	**CYP2D6 inhibitor**	No
Muegge #violations	1	**CYP3A4 inhibitor**	No
Bioavailability Score	0.55	**log Kp (cm/s)**	−4.43
PAINS #alerts	0	**Lipinski #violations**	1
Brenk #alerts	0	**Ghose #violations**	1
Leadlikeness #violations	2	**Veber #violations**	0

involving PIK3CA and BCL2 genes. Further, a strong binding affinity of tomatidine to these genes within these pathways strengthen rationale to target the genes with drugs. This is further substantiated by Our findings where tomatidine showed does dependent reduction in KB cell survival. Therefore, tomatidine has strong potential use as an adjunct to conventional chemo or radiotherapy in OSCC.

Even though our study reported that tomatidine suppresses PI3K/Akt signaling to induce Apoptosis in KB cell lines, there are certain limitations. First, considering the in-vitro study design and computation nature of analysis, it lacks classical RCT design features such as randomizations and prospective nature. Second, the molecular docking indicates potential binding of tomatidine to key signaling proteins, yet functional inhibition in KB cells was not confirmed through western blotting or apoptotic assay. It is recommended to conduct future studies to confirm these findings on animal models. Finally, by modulating PI3K/Akt pathways and exerting effects toward oxidative stress, tomatidine emerges as a promising low-toxicity, multi-targeted anticancer agent. Future studies examining tomatidine's modulation of the PI3K-Akt signaling cascade may establish tomatidine as a viable candidate for the treatment of OSCC.

5. Conclusion

The present study demonstrates that tomatidine exerts significant anticancer effects against OSCC cells by inhibiting cell viability and promoting apoptosis. The dose-dependent cytotoxicity observed in KB cells indicates its potential effectiveness as a natural therapeutic agent. Molecular docking further supports these findings by revealing strong binding affinities of tomatidine to key regulators of the PI3K/Akt pathway, including PI3K, AKT, and PTEN, as well as apoptosis-related proteins such as BCL-2 and Caspase-3. These interactions suggest that tomatidine may effectively interfere with the PI3K/Akt signaling cascade, contributing to the induction of programmed cell death. Overall, the results highlight tomatidine's promising role as a potential anticancer compound for OSCC, warranting further in-depth studies, including molecular validation and *in vivo* investigations, to fully elucidate its therapeutic efficacy and mechanism of action.

Patient's/Guardian's consent

It not applicable.

Ethical clearance

The present study was conducted following the ethical standards of the institutional research committee and in accordance with the Declaration of Helsinki. Ethical approval for this study was obtained from the Institutional Human Ethical Committee (IHEC), under the approval number IHEC/SDC/UG-2384/25/GPATH/112.

As this research involved only in vitro cell culture and bioinformatics analyses, no human participants or animal models were directly involved.

Data availability statement

The author confirms that all data are included in this published article.

Ethical clearance

IHEC/SDC/UG-2384/25/GPATH/112.

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Declaration of competing interest

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

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References

1. Coletta RD, Yeudall WA, Salo T. Current trends on prevalence, risk factors and prevention of oral cancer. *Front Oral Health*. 2024;5, 1505833.
2. Muthusamy M, Ramani P, Arumugam P, et al. Assessment of various etiological factors for oral squamous cell carcinoma in non-habit patients-a cross-sectional case control study. *BMC Oral Health*. 2025;25(1):62.

3. Dong L, Xue L, Cheng W, Tang J, Ran J, Li Y. Comprehensive survival analysis of oral squamous cell carcinoma patients undergoing initial radical surgery. *BMC Oral Health*. 2024;24(1):919.
4. Ramalingam K, Yadalam PK, Ramani P, et al. Light gradient boosting-based prediction of quality of life among oral cancer-treated patients. *BMC Oral Health*. 2024;24(1):349.
5. Stawarz K, Bieńkowska-Pluta K, Galazka A, et al. Clinicopathologic predictors of survival following oral cancer surgery: a retrospective cohort study. *Cancers*. 2025;17(15):2454.
6. Xu Z, Xu M, Sun Z, Feng Q, Xu S, Peng H. A nomogram for predicting overall survival in oral squamous cell carcinoma: a SEER database and external validation study. *Front Oncol*. 2025;15, 1557459.
7. R Kumar A, Kannan B, Girija As S, Jayaseelan VP, Arumugam P. Aberrant promoter methylation of CTHRC1 gene and its clinicopathological characteristics in head and neck cancer. *Int J Oral Maxillofac Surg*. 2025;54(7):581–589.
8. Longfei H, Shangyin Z, Ju Z. Outcomes of postoperative radiotherapy combined with or without chemotherapy for locally advanced oral cancer: a systematic review and meta-analysis. *J Oral Maxillofac Surg Med Pathol*. 2025;37(1):141–150.
9. Yellarthi PK, Muthukrishnan A, Rao GR, Pavankumar S, Uppala D. A systematic review on the role of desmoglein 1 and desmoglein 3 in etiopathogenesis and metastasis of oral cancer. *J Indian Acad Oral Med Radiol*. 2025;37(2):129–136.
10. Lim B, Greer Y, Lipkowitz S, Takebe N. Novel apoptosis-inducing agents for the treatment of cancer, a new arsenal in the toolbox. *Cancers*. 2019;11(8):1087.
11. Kumar RR, Kannan B, Pandi C, Pandi A, Jayaseelan VP, Arumugam P. Dysregulation of a novel m6A regulator YWHAG is correlated with metastasis and poor prognosis in oral squamous cell carcinoma–A cross-sectional study. *Arch Oral Biol*. 2025;169, 106090.
12. Townsend PA, Kozhevnikova MV, Cexus ON, Zamyatnin Jr AA, Soond SM. BH3-mimetics: recent developments in cancer therapy. *J Exp Clin Cancer Res*. 2021;40(1):355.
13. Cháirez-Ramírez MH, de la Cruz-López KG, García-Carrancá A. Polyphenols as antitumor agents targeting key players in cancer-driving signaling pathways. *Front Pharmacol*. 2021;12, 710304.
14. Li C, Jia WW, Yang JL, Cheng C, Olaleye OE. Multi-compound and drug-combination pharmacokinetic research on Chinese herbal medicines. *Acta Pharmacol Sin*. 2022;43(12):3080–3095.
15. Ngo TH, Park J, Jo YD, et al. Content of two major steroidal glycoalkaloids in tomato (Solanum lycopersicum cv. micro-tom) mutant lines at different ripening stages. *Plants*. 2022;11(21):2895.
16. Imbesi Bellantoni M, Picciolo G, Pirrotta I, et al. Oral cavity squamous cell carcinoma: an update of the pharmacological treatment. *Biomedicines*. 2023;11(4):1112.
17. Chihomvu P, Ganesan A, Gibbons S, Woollard K, Hayes MA. Phytochemicals in drug discovery—A confluence of tradition and innovation. *Int J Mol Sci*. 2024;25(16):8792.
18. Mukherjee D, Chakraborty S, Bercz L, et al. Tomatidine targets ATF4-dependent signaling and induces ferroptosis to limit pancreatic cancer progression. *iScience*. 2023;26(8), 107408.
19. Wu H, Li W, Wang T, et al. α-Tomatine, a novel early-stage autophagy inhibitor, inhibits autophagy to enhance apoptosis via Beclin-1 in Skov3 cells. *Fitoterapia*. 2021;152, 104911.
20. Ghafouri-Fard S, Noie Alamdari A, Noee Alamdari Y, et al. Role of PI3K/AKT pathway in squamous cell carcinoma with an especial focus on head and neck cancers. *Cancer Cell Int*. 2022;22(1):254.
21. Rajendran P, Sekar R, Dhayasankar PS, et al. PI3K/AKT signaling pathway mediated autophagy in oral carcinoma-a comprehensive review. *Int J Med Sci*. 2024;21(6):1165.
22. Starzyńska A, Sejda A, Adamska P, et al. Prognostic value of the PIK3CA, AKT, and PTEN mutations in oral squamous cell carcinoma: literature review. *Arch Med Sci*. 2020;17(1):207.
23. Friedman M, Levin CE, Lee SU, et al. Tomatine-containing green tomato extracts inhibit growth of human breast, colon, liver, and stomach cancer cells. *J Agric Food Chem*. 2009;57(13):5727–5733.
24. Yan KH, Lee LM, Yan SH, et al. Tomatidine inhibits invasion of human lung adenocarcinoma cell A549 by reducing matrix metalloproteinases expression. *Chem Biol Interact*. 2013;203(3):580–587.
25. Fujimaki J, Sayama N, Shiotani S, et al. The steroidal alkaloid tomatidine and tomatidine-rich tomato leaf extract suppress the human gastric cancer-derived 85As2 cells in vitro and in vivo via modulation of interferon-stimulated genes. *Nutrients*. 2022;14(5):1023.
26. Hsieh MH, Yang JS, Lin RC, et al. Tomatidine represses invasion and migration of human osteosarcoma U2OS and HOS cells by suppression of presenilin 1 and C RAF–MEK–ERK pathway. *Molecules*. 2020;25(2):326.
27. Wu Y, Wang Y, Liu H, et al. Mechanism of apoptosis in oral squamous cell carcinoma promoted by cardamonin through PI3K/AKT signaling pathway. *Sci Rep*. 2024;14(1), 20802.
28. Veerasamy V, Veeran V, Nagini S. Dysregulated PI3K/AKT signaling in oral squamous cell carcinoma: the tumor microenvironment and epigenetic modifiers as key drivers. *Oncol Res*. 2025;33(8):1835.
29. Wang X, Wang J, Hua H, et al. Geniposide inhibits oral squamous cell carcinoma by regulating PI3K-Akt signaling-mediated apoptosis: a multi-method validation study. *Curr Issues Mol Biol*. 2025;47(9):786.
30. Pathoor NN, Ganesh PS. Unveiling the nexus: long non-coding RNAs and the PI3K/Akt pathway in oral squamous cell carcinoma. *Pathol Res Pract*. 2024;262, 155540.
31. Tashiro K, Oikawa M, Miki Y, Takahashi T, Kumamoto H. Immunohistochemical assessment of growth factor signaling molecules: MAPK, Akt, and STAT3 pathways in oral epithelial precursor lesions and squamous cell carcinoma. *Odontology*. 2020;108(1):91–101.
32. Zhang X, Xu Y, Shi L, et al. FGF6 inhibits oral squamous cell carcinoma progression by regulating PI3K/AKT and MAPK pathways. *Sci Rep*. 2024;14(1), 26877.
33. Glaviano A, Foo AS, Lam HY, et al. PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. *Mol Cancer*. 2023;22(1):138.
34. Wang X, Wang J, Hua H, et al. Geniposide inhibits oral squamous cell carcinoma by regulating PI3K-Akt signaling-mediated apoptosis: a multi-method validation study. *Curr Issues Mol Biol*. 2025;47(9):786.
35. Glaviano A, Foo ASC, Lam HY, et al. PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. *Mol Cancer*. 2023;22(1):138.
36. Yu CC, Hung SK, Lin HY, et al. Targeting the PI3K/AKT/mTOR signaling pathway as an effectively radiosensitizing strategy for treating human oral squamous cell carcinoma in vitro and in vivo. *Oncotarget*. 2017;8(40), 68641.