

Apigenin and Carbenoxolone Co-Treatment Induces Potent Cytotoxicity in CAL-27 Oral Squamous Cell Carcinoma: A Morphological and Dose-Response Study

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ABSTRACT

Background: Oral squamous cell carcinoma is a major health burden across the globe due to its widespread incidence, limited effective therapeutics, and high recurrence rates. Natural and synthetic compounds such as Apigenin (API) and Carbenoxolone (CBX) have shown significant antitumor potential against various cancer types. The current study aimed to evaluate the anti-proliferative potential of API and CBX, alone or in combination in the CAL-27 oral cancer cell line.

Methods: MTT assay was used to analyze the cytotoxic potential of API, CBX, and their combination in the CAL-27 cell line. Differential Interference Contrast (DIC) microscopy was performed to identify alterations in cellular morphology after treatment.

Results: The half maximal inhibitory concentrations for API and CBX were calculated as 50 μ M and 200 μ M respectively. Significantly greater inhibition of 86.37% (p-value <0.001) was observed after combination treatment in the CAL-27 cell line. Microscopic examination revealed cell death, cell shrinkage and detachment, and membrane damage in combination-treated cells compared with control and single agent treatment groups.

Conclusion: Treatment with API and CBX exhibited decreased cell viability, and their combination further increased anti-proliferative effects in CAL-27 cells, suggesting that these compounds in combination are potential candidates for anticancer therapy in OSCC.

Keywords: Apigenin, Carbenoxolone, Cytotoxicity, Combination therapy, Cytology, Oral squamous cell carcinoma.

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INTRODUCTION

Oral Squamous Cell Carcinoma (OSCC) is one of the most prevalent malignancies of the oral cavity with significant impact on the global health burden, especially in the South Asia, where its prevalence continues to rise at a substantial rate.^{1,2} Socioeconomic disparities, tobacco consumption, betel nut use, alcohol exposure, poor oral hygiene, poor nutritional status, and infection with tumorigenic HPV genotype strongly influence disease impact.³⁻⁴

Moreover, demographic transitions underscore a significant disparity and pronounced rise in OSCC within the male population, younger age groups, and underserved population, across intraoral regions, including buccal, labial mucosa, alveolar ridge, tongue, hard palate, retromolar trigone, and floor of the mouth.^{5,6} Oral cancer has been shown to exhibit drug resistance towards various chemotherapeutic drugs, resulting in decreased treatment efficacy. Hence, there is a dire need to develop more effective and novel anticancer agents. While



advancements in surgical and anticancer therapy continue, the treatment efficacy remains insufficient with undesired side effects, which stresses the necessity for developing newer therapies with optimized safety and efficacy profiles.

Bioactive phytochemicals have drawn interest as putative anticancer agents owing to their biodiversity and lower toxicity. Flavonoids comprise a key category of phytochemicals with anticancer properties.^{7,8} Apigenin (API), a naturally derived flavonoid, has been extensively documented to attenuate cancer cell proliferation and enhance tumor cell death across diverse cancer types.⁹ Its tumor-suppressive activity has been linked with restriction of cellular growth, attenuation of metastatic capacity, and induction of structural and morphological alterations in malignant cells by disrupting key signaling pathways such as PI3K/Akt, MAPK, and p53.¹⁰

Carbenoxolone (CBX), a derivative of glycyrrhetic acid extracted from the liquorice plant and a gap junction inhibitor, is known to have a broad pharmacological profile, including anti-inflammatory, antiviral, anticancer, antibacterial, anti-fibrotic, and neuroprotective.^{11,12} As proven from the previous studies that showed that CBX can decrease the cell viability by inducing apoptosis in multiple cancer cell lines.¹³⁻¹⁵

Although API and CBX have individually demonstrated anticancer activity in various malignancies, comparative studies examining their cytotoxic effects alongside morphological changes in OSCC cell lines are scarce. Combining both compounds can enhance their anticancer effect by simultaneously inhibiting proliferation and inducing cell death. Additionally, CBX-induced membrane and mitochondrial destabilization may sensitize cancer cells to API-mediated anti-proliferative signaling, resulting in synergistic or additive anticancer effects in OSCC. CAL-27 (tongue squamous cell carcinoma) cell line used in this study was selected because it is one of the most reported cell lines in the literature and is well-

characterized at the molecular level. Compared to other oral cancer cell lines, it provides stable proliferation kinetics and reliable dose-response outcomes. CAL-27 exhibits an overactive PI3K/AKT pathway and is a suitable cell line for the investigation of cytotoxicity, proliferation, and migration.^{16,17} Hence, the current study aimed to evaluate the cytotoxic effects of API and CBX in the OSCC (CAL-27) cell line using the MTT assay and to characterize treatment-induced morphological alterations via DIC microscopy.

METHODOLOGY

Cell Culture

The human oral squamous cell carcinoma cell line CAL-27 was used in this study. It was an in vitro experimental study conducted at Multidisciplinary Research Lab 1 (MDRL-1), Ziauddin University, Karachi, Pakistan. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Thermo Fisher Scientific, USA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were cultured at 37 °C in a humidified incubator with 5% CO₂ and sub-cultured at 80-90% confluency. Cells were treated with trypsin-EDTA. The cell line at passage number 7 was used for the study. The cell line was previously tested for mycoplasma contamination using DAPI staining and was found to be mycoplasma negative.

Reagents

API (A167323) and CBX (C4790) were obtained from Sigma- Aldrich (USA). Trypsin-EDTA (25200-056), Dulbecco's Modified Eagle Medium (11995-065), Foetal Bovine Serum (10270-106), Dimethyl Sulfoxide (137285925), MTT dye (M6494), Phosphate Buffered Saline (10010023), and Penicillin-Streptomycin (15070063) were obtained from Thermo Fisher Scientific (USA).

Cell Viability Assay

Cell viability was assessed using the MTT assay. CAL-27 cells were plated in 96-well plates at a density of 8×10^3 cells per well, and subjected to overnight incubation for cell

adherence. Different treatment concentrations of CBX (300 μ M, 150 μ M, 75 μ M) and API (200 μ M, 100 μ M, 50 μ M, 25 μ M) were added. Combination treatment was performed using half maximal inhibitory concentrations of 50 μ M for API and 200 μ M for CBX for 48 hours. Following compound exposure, the MTT solution (0.5 mg/mL) was dispensed into each well and cultured for 3 h at 37°C to allow formation of formazan crystals. Removal of cell culture media was done, and crystals were solubilized in Dimethyl Sulfoxide (DMSO). To rule out vehicle (DMSO) induced cytotoxicity, vehicle control wells received the same amount of DMSO as the compound-treated wells. Furthermore, the final concentration of DMSO was $\leq 0.25\%$ across all treatment groups, which is below the cytotoxic threshold for cell lines. Absorbance was determined at 570 nm using a MultiSkan Sky microplate reader. Relative cell viability was calculated using untreated control cells as a baseline. All experiments were conducted in triplicate and replicated independently three times.

Coefficient of Drug Interaction (CDI) for the half maximal inhibitory concentrations of 50 μ M for API and 200 μ M for CBX was determined by using fractional viability using the percent inhibition formula:

- **Fractional Viability** = $1 - (\text{Cell Death (\%)} / 100)$
- **CDI** = $\text{Combination of Drug A \& B} / \text{Drug A} \times \text{Drug B}$

Differential Interference Contrast (DIC) Microscopy

The cells were plated at a density of 50,000 cells per well in a 24-well plate. Cells were incubated for 24 hours before treatment to induce morphological changes. Afterwards, the compounds API, CBX, and their co-treatment were introduced for 48 hours. Post treatment, cells were rinsed with PBS, and DIC microscopy was used to assess and document morphological changes. Imaging was done using a Nikon Eclipse Ts2 microscope at 10x

magnification using Nikon NIS-Elements imaging software.

Statistical Analysis

Analysis was done using SPSS version 24.0. The graphs were made using the same software. Percent inhibition in the control, API, CBX, and co-treatment groups was expressed as mean $\pm$ standard deviation. Normality of the data distribution was assessed using the Shapiro-Wilk test. For comparison among multiple groups, one-way analysis of variance (ANOVA) was applied, followed by Tukey's post hoc test for pairwise comparison, along with 95% confidence interval (CI) for the estimation of the magnitude and precision of the treatment effects. A p-value of less than 0.05 was considered significant.

RESULTS

API and CBX Reduce CAL-27 Cell Viability in a Dose-Dependent Manner

Viability of CAL-27 cells following treatment with API and CBX was evaluated using the MTT assay. Both compounds exhibited a reduction in cell viability in a dose-dependent manner as compared to untreated (DMSO vehicle control) CAL-27 cells.

Treatment with API resulted in growth inhibition of approximately 11.52 ± 3.99 (95%CI=1.58-21.45), 51.16 ± 1.850 (95%CI=46.57-55.76), 69.23 ± 1.950 (95%CI= 64.38-74.07), and 70.48 ± 2.17 (95%CI= 65.08-75.88) at 25 μ M, 50 μ M, 100 μ M, and 200 μ M, respectively, after 48 hours (Figure 1A). These doses and times were selected after a literature review and a pilot MTT assay. Approximately 50% inhibition of cell viability was observed at 50 μ M, indicating the half-maximal inhibitory concentration of API in CAL-27 cells.

CBX treatment also induced a dose-dependent reduction in cell viability. Growth inhibition values of approximately 3.1652 ± 2.20 (95%CI=-2.31-8.65), 10.39566 ± 0.52 , (95%CI= 9.09-11.69), and 80.6341 ± 3.60 , (95%CI= 71.69-89.57) were observed at 75 μ M, 150 μ M, and

300 μ M (Figure 1B). Concentrations below 75 μ M did not inhibit the cell growth, and were therefore not reported. The estimated half-maximal inhibitory concentration of CBX was 200 μ M. To evaluate potential combined effects, CAL-27 cells were co-treated with API (50 μ M) and CBX (200 μ M). This combination treatment produced the greater reduction in cell viability of

86.37 $\pm$ 1.40 (95%CI= 82.87-89.85), exceeding the inhibitory effects observed with individual treatments (Figure 1C). The combination of API and CBX half maximal inhibitory concentration showed synergistic/enhanced effect after calculation (Table 1). The statistical analysis for inter and intra group comparisons and multiple comparisons between compounds are depicted in Table 2 and 3.

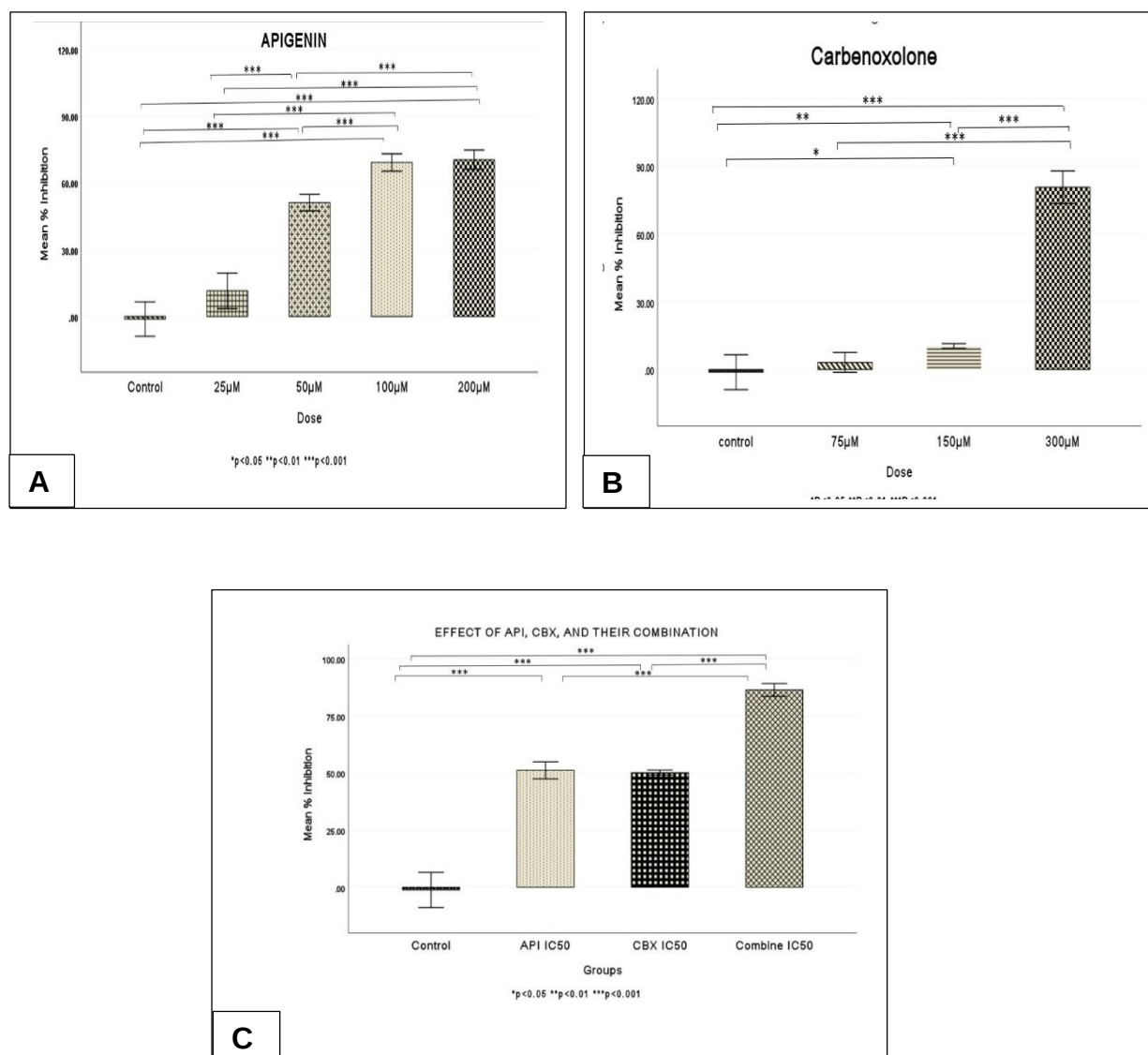


Figure 1: Dose-dependent cytotoxicity effects of Carbenoxolone, Apigenin, and their combination in CAL-27 cells (A)percentage inhibition at varying API concentrations (B)percentage inhibition at varying CBX concentrations, (C) percentage inhibition at the half maximal inhibitory concentration of API, CBX, and their combination, with maximal inhibition observed at combine half maximal inhibitory concentration. Data are presented as mean $\pm$ SD of three independent experiments. Statistical analysis was performed using One-way ANOVA followed by Tukey's post hoc multiple comparison test. Differences compared among various groups were observed at $p < 0.05$, $p < 0.01$, and $p < 0.001$.

Table-1: Coefficient of Drug Interaction (CDI) Analysis Using Fractional Viability

Combination Dose	% Cell Death	% Cell Viability	Fractional Viability	CDI Values	Interpretation
CBX 200µM	50%	50%	0.50	0.558	Synergistic Effect
API 50µM	51.16%	48.84%	48.84		

Table-2 One-Way ANOVA Analysis of Apigenin and Carbenoxolone-Induced Inhibition

ANOVA (APIGENIN)			
	df	F-statistic	Significance
Between Groups	4	386.626374	<.001
Within Groups	10		
Total	14		
ANOVA (Carbenoxolone)			
	df	F-statistic	Sig.
Between Groups	3	540.801070	<.001
Within Groups	8		
Total	11		

Table-3 Multiple Comparison for Apigenin and Carbenoxolone (Post Hoc Tukey Test)

Group-wise Comparison			Significance
Apigenin	Control	API 25 µM	0.002
		API 50 µM	<.001
		API 100 µM	<.001
		API 200 µM	<.001
	25 µM	API 50 µM	<.001
		API 100 µM	<.001
		API 200 µM	<.001
	50 µM	API 100 µM	<.001
		API 200 µM	<.001
Carbenoxolone	Control	CBX 150 µM	0.005
		CBX 300 µM	<.001
	75 µM	CBX 300 µM	<.001
	150 µM	CBX 300 µM	<.001

Morphological Alterations Observed by DIC Microscopy

DIC microscopy was used to evaluate the significant morphological changes in the CAL-27 cell line, which showed a notable difference between the treated and untreated groups. In the untreated group (control), the cells displayed typical epithelial morphology, with adherent cells and cellular contact, all in a healthy, proliferative condition without any cellular stress (Figure 2A). On the other hand, the API-treated group showed enhanced morphological changes, indicating decreased cellular health, including early signs of cell death, cell rounding, cellular shrinkage, cytoplasmic condensation, membrane disruption, and cell detachment from the cell culture surface (Figure 2B).

Likewise, CBX-treated cells showed marked cellular changes (Figure 2C). Signs of cellular stress, including cell rounding, irregular cell boundaries, reduced cell count, and loss of adhesion to the cell culture surface, indicate cell death. Remarkably, combined treatment with API and CBX (Figure 2D) appeared to cause the most pronounced morphological damage among all groups. The cells showed extensive damage to cell membranes, loss of adherence, and pronounced cytoplasmic shrinkage. Cellular fragmentation and lysis were also observed post-treatment, indicating cell death. All treatment groups exhibited cytotoxic effects, however the most notable decrease in cell viability and structural integrity was observed in the combination treatment group as compared to the control.

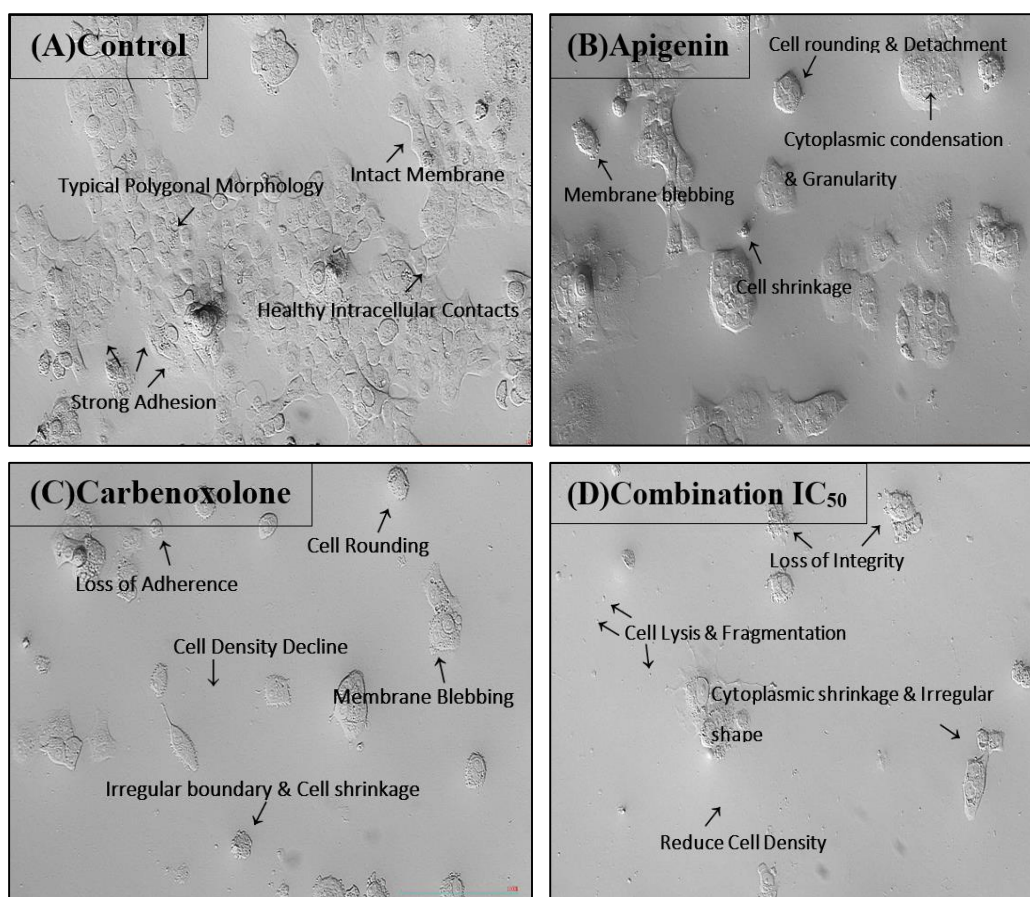


Figure 2: CAL-27 cells after treatment for 48 hours as observed by differential interference contrast (DIC) microscopy. Cells treated with API, CBX, and their combination displayed cytotoxic effects, scale bar 50µm.

DISCUSSION

API is widely considered a natural compound and a flavonoid widely considered as anticancer. The research findings of this study are consistent with results reported in the literature showing a dose-dependent effect. Decreased HSC-3 tongue carcinoma cell line proliferation following API exposure demonstrated its capacity to inhibit tumor cell growth even at comparatively low concentrations. In gastric carcinoma cell lines, API was found to decrease cell viability via dysregulating cell survival pathways and induction of apoptosis. The similar inhibitory pattern seen in this study is aligned with the broad anticancer activity of API against epithelial cancers.¹⁸

Comparatively lower sensitivity was observed in the CBX group against the CAL-27 cell line. Similar findings were also observed against K562 leukemia cells, where the compound induced proliferation reduction at around 150 μ M concentration. The comparatively higher half-maximal inhibitory concentration observed in the CBX group in this study may reflect tumor-specific responses toward drug treatment, influenced by cancer type and molecular background.¹⁹ In another study, CBX exhibited anticancer effects against small cell lung carcinoma (SCLC) cells at concentrations ranging from 0-100 μ M by targeting pannexin 1, which in turn suppressed the extracellular ATP levels.²⁰

The combination therapy showed enhanced efficacy compared with individual groups, indicating that combination strategies targeting complementary cellular pathways are effective approaches in cancer therapy. API and CBX target complementary oncogenic pathways and cause enhanced cell death. As per evidence that, API targets the cancer cell survival pathways such as PI3K/AKT, and CBX acts at the intercellular level by inhibiting gap junctions, simultaneously targeting intracellular survival pathways and intercellular communication, and metabolic pathways can produce a multi-targeted anticancer effect.^{21,22}

Despite the antitumor potential of API and CBX, their efficacy across different cancers varies. For instance, an API's ability to induce apoptosis can vary depending on signaling pathway modulations within specific tumors.²³ Similarly, the differences in sensitivity observed with CBX at high concentrations in certain suspension cancer cell lines, compared with lower sensitivity in solid tumors, highlight tumor-specific effects. CBX can also function as a channel blocker, suppressing cancer proliferation.²⁴

Morphological evaluation further supported the cytotoxic findings. Untreated CAL-27 cells maintained epithelial morphology, whereas treated cells displayed structural alterations consistent with cellular stress and death. Similar morphological changes following API treatment have been reported in endometrial carcinoma cells, in which cell shrinkage, loss of adhesion, and reduced spreading were associated with apoptotic processes.²⁵ The same morphological alteration was seen in the leukemia model after treatment of the leukemic cells with CBX¹⁹. In the combination treatment group, the most significant structural damage was seen which intensified cellular damage. Therefore, supporting the findings of reduced cell viability in the MTT assay.

The selective toxicity is crucial for the creation of anticancer drugs. The prior experimental evidence supports API's minimal cytotoxicity in non-malignant cellular systems, and it was seen in the retina of rats both in vivo and in vitro.²⁶ Similar effects of CBX have been reported to modulate intracellular signaling in normal endothelial cells without inducing irreversible cytotoxic damage.²⁷ These findings show the potential therapeutic relevance of API and CBX as supplementary anti-tumor candidates with acceptable safety profiles, although confirmation in OSCC-specific normal cell models remains necessary.

Collective findings of the present study suggest that API functions as a potent growth inhibitor in OSCC cells, while CBX contributes additional cytotoxic stress that enhances overall treatment

efficacy when used in combination. Such multi-agent strategies may represent a promising approach for improving therapeutic responses in oral cancer while minimizing drug-associated cytotoxicity.

Limitations

The use of a single OSCC cell line may not fully reflect the effect of compounds, and results may not apply to other oral cancer subtypes. Furthermore, the study lacks critical molecular analysis, such as apoptotic evaluation, ROS production studies, and gene expression analysis. These parameters could have provided a deeper understanding of the molecular mechanism of action of these compounds. Furthermore, in vivo animal studies were also not conducted.

CONCLUSION

This study highlights the significant anticancer effects of API and CBX in the CAL-27, a representative cell line of OSCC. API exhibited higher potency as compared to CBX, and their combination resulted in enhanced anti-proliferative effects. Combination treatment also resulted in structural alterations as well as significant cell death, as evident by microscopic examination. These findings support the combined therapeutic potential of API and CBX in OSCC.

Author Contributions

AM: Conception & Design, Data Collection, Data Analysis & Interpretation, Manuscript Writing, Critical Revision and Final Approval

RI: Conception & Design, Data Collection, Data Analysis & Interpretation, Critical Revision and Final Approval

SH: Critical Revision and Final Approval

AB: Critical Revision and Final Approval

AH: Data Analysis & Interpretation, Critical Revision and Final Approval

Ethical Approval

This study is approved by the Ethical Review Committee of Ziauddin University, Karachi, Pakistan (**Ref: 7740923ABPHY**)

Conflict of Interests

The authors proclaim no potential bias.

Data Availability

Data will be available upon request.

Funding Source

No sources.

REFERENCES

1. Nokovitch L, Maquet C, Frédéric Crampon, Ihsène Taihi, Roussel LM, Rais Obongo, et al. Oral Cavity Squamous Cell Carcinoma Risk Factors: State of the Art. Oral Cavity Squamous Cell Carcinoma Risk Factors: State of the Art [Internet]. 2023 May 3;12(9):3264. Available from: <https://doi.org/10.3390/jcm12093264>
2. Deshmukh V, Shekar K. Oral Squamous Cell Carcinoma: Diagnosis and Treatment Planning. Oral and Maxillofacial Surgery for the Clinician [Internet]. 2021;12(9):1853–67. Available from: https://doi.org/10.1007/978-981-15-1346-6_81
3. Givony S. Oral squamous cell carcinoma (OSCC) an overview. Journal of Medical Sciences. 2020;8(13):67–74.
4. Kalogirou EM, Tosios KI, Christopoulos PF. The Role of Macrophages in Oral Squamous Cell Carcinoma. Frontiers in Oncology [Internet]. 2021 Mar 18;11:611115. Available from: <https://doi.org/10.3389/fonc.2021.611115>
5. Coletta RD, Yeudall WA, Salo T. Grand Challenges in Oral Cancers. Frontiers in Oral Health [Internet]. 2020 Jun 9;1. Available from: <https://doi.org/10.3389/froh.2020.00003>
6. Miranda-Filho A, Bray F. Global patterns and trends in cancers of the lip, tongue and mouth. Oral Oncology [Internet]. 2020 Mar [cited 2020 Feb 10];102:104551. Available from: <https://doi.org/10.1016/j.oraloncology.2019.104551>
7. Constantinescu T, Lungu CN. Anticancer Activity of Natural and Synthetic Chalcones. International Journal of Molecular Sciences [Internet]. 2021 Oct 20;22(21):11306. Available from: <https://doi.org/10.3390/ijms222111306>
8. Imran M, Aslam Gondal T, Atif M, Shahbaz M, Batool Qaisarani T, Hanif Mughal M, et al. Apigenin as an anticancer agent. Phytotherapy Research [Internet]. 2020 Feb 14;34(8):1812–28. Available from: <https://doi.org/10.1002/ptr.6647>
9. Rahmani AH, Alsahli MA, Almatroudi A, Almogbel MA, Khan AA, Anwar S, et al. The Potential Role of Apigenin in Cancer Prevention and Treatment. Molecules [Internet]. 2022 Sep 16;27(18):6051. Available from: <https://doi.org/10.3390/molecules27186051>
10. Naponelli V, Maria Teresa Rocchetti, Mangieri D. Apigenin: Molecular Mechanisms and Therapeutic Potential against Cancer Spreading. International Journal of Molecular Sciences [Internet]. 2024 May 20;25(10):5569–9. Available from: <https://doi.org/10.3390/ijms25105569>
11. Sharma S, Saini A, Nehru B. Neuroprotective effects of carbenoxolone against amyloid-beta 1–42 oligomer-induced neuroinflammation and cognitive decline in rats. NeuroToxicology [Internet]. 2021 Mar [cited 2021 Nov 24];83:89–105. Available from: <https://doi.org/10.1016/j.neuro.2020.12.015>
12. Zhang L Z, Fan Z, Wang L, Liu L, Li X, Li L, et al. Carbenoxolone decreases monocrotaline induced pulmonary inflammation and pulmonary arteriolar remodeling in rats by decreasing the expression of connexins in T lymphocytes. International Journal of Molecular Medicine [Internet]. 2019 Nov 13 [cited 2025 Nov 24];45(1):81–92. Available from: <https://doi.org/10.3892/ijmm.2019.4406>
13. Salvi M, Fiore C, Battaglia V, Palermo M, Armanini D, Toninello A. Carbenoxolone Induces Oxidative Stress in Liver Mitochondria, Which Is Responsible for

- Transition Pore Opening. *Endocrinology* [Internet]. 2005 May 1 [cited 2020 Dec 14];146(5):2306–12. Available from: <https://doi.org/10.1210/en.2004-1128>
14. Song Z, Zhao C, Yan J, Jiang D, Jia G. Carbenoxolone Disodium Suppresses the Migration of Gastric Cancer By Targeting HDAC6. *Future Medicinal Chemistry* [Internet]. 2023 Feb;15(4):333–44. Available from: <https://doi.org/10.4155/fmc-2022-0246>
 15. Tamborini M, Valentino Ribecco, Elisabetta Stanzani, Sironi A, Tambalo M, Franzone D, et al. Extracellular Vesicles Released by Glioblastoma Cancer Cells Drive Tumor Invasiveness via Connexin-43 Gap Junctions. *Neuro-Oncology* [Internet]. 2025 Jan 30 [cited 2025 Sep 14];27(11):2843–60. Available from: <https://doi.org/10.1093/neuonc/noaf013>
 16. Lai J, Fang C, Zhang G, Shi C, Yu F, Gu W, et al. Novel Prognostic Model Construction of Tongue Squamous Cell Carcinoma Based on Apigenin-Associated Genes. *Frontiers in Bioscience-Landmark* [Internet]. 2024 Feb 6 [cited 2026 May 16];29(2):65. Available from: <https://doi.org/10.31083/j.fbl2902065>
 17. Kim TW, Lee HG. Apigenin Induces Autophagy and Cell Death by Targeting EZH2 under Hypoxia Conditions in Gastric Cancer Cells. *International Journal of Molecular Sciences* [Internet]. 2021 Dec 15;22(24):13455. Available from: <https://doi.org/10.3390/ijms222413455>
 18. Pei C, Zhang J, Li J, Zhou D. Apigenin suppresses the low oxaliplatin-induced epithelial-mesenchymal transition in oral squamous cell carcinoma cells via LINC00857. *Translational Cancer Research* [Internet]. 2024 May;13(5):2164–74. Available from: <https://doi.org/10.21037/tcr-23-2335>
 19. Moosavi M, S Moasses Ghafary, I Asvadi-Kermani, H Hamzeiy, Rahmati M, Ahmadi A, et al. Carbenoxolone induces apoptosis and inhibits survivin and survivin-ΔEx3 genes expression in human leukemia K562 cells. *DARU Journal of Pharmaceutical Sciences* [Internet]. 2026;19(6):455. Available from: <https://pmc.ncbi.nlm.nih.gov/articles/PMC3436083/>
 20. Tsuruda T, Kodama M, Nakayama KI. Small Cell Lung Cancer Establishes a Metabolic Autocrine Mechanism Through Active Extracellular ATP Transport. *Cancer Science* [Internet]. 2026 Mar 2;117(5):1333–45. Available from: <https://doi.org/10.1111/cas.70350>
 21. Edwards E, Schenone D, Sivagnanalingam U, Perry S, Mullen Ca. Gap Junction Function is Essential for Survival of Acute Lymphoblastic Leukemia Cells. *Experimental Oncology* [Internet]. 2024 Oct 9 [cited 2026 May 16];46(2):110–8. Available from: <https://doi.org/10.15407/exp-oncology.2024.02.110>
 22. Chen YH, Wu JX, Yang SF, Yang CK, Chen TH, Hsiao YH. Anticancer Effects and Molecular Mechanisms of Apigenin in Cervical Cancer Cells. *Cancers* [Internet]. 2022 Apr 4;14(7):1824. Available from: <https://doi.org/10.3390/cancers14071824>
 23. Fossatelli L, Zaira Maroccia, Fiorentini C, Massimo Bonucci. Resources for Human Health from the Plant Kingdom: The Potential Role of the Flavonoid Apigenin in Cancer Counteraction. *International journal of molecular sciences* [Internet]. 2023 Dec 23;25(1):251–1. Available from: <https://doi.org/10.3390/ijms25010251>
 24. Langlois S, Marie-Eve St-Pierre, Holland SH, Xiang X, Freeman E, Mohamed H, et al. Inhibition of PANX1 Channels Reduces the Malignant Properties of Human High-Risk Neuroblastoma. *Journal of Cancer* [Internet]. 2023 Jan 1;14(5):689–706. Available from: <https://doi.org/10.7150/jca.79552>
 25. Liang YC, Zhong Q, Ma RH, Ni ZJ, Thakur K, Mohammad Rizwan Khan, et al. Apigenin inhibits migration and induces apoptosis of human endometrial carcinoma Ishikawa cells via PI3K-AKT-GSK-3β pathway and endoplasmic reticulum stress. *Journal of Functional Foods* [Internet]. 2022 Jul 1 [cited 2024 Mar 11];94. Available from: <https://doi.org/10.1016/j.jff.2022.105116>
 26. Wu J, Zhang D, Liu H, Li J, Li T, Wu J, et al. Neuroprotective effects of apigenin on retinal ganglion cells in ischemia/reperfusion: modulating mitochondrial dynamics in vivo and in vitro models. *Journal of Translational Medicine* [Internet]. 2024 May 13;22(1). Available from: <https://doi.org/10.1186/s12967-024-05260-1>
 27. Buckley C, Zhang X, Wilson C, McCarron JG. Carbenoxolone and 18β-glycyrrhetic acid inhibit inositol 1,4,5-trisphosphate-mediated endothelial cell calcium signalling and depolarise mitochondria. *British Journal of Pharmacology* [Internet]. 2021 Jan 17 [cited 2023 Dec 14];178(4):896–912. Available from: <https://doi.org/10.1111/bph.15329>