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Original article

Assessment of the Anticancer Effect of Polyphenol Extract from Olive Oil on Oral Squamous Cell Carcinoma Cell Lines: An *in vitro* Study

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Abstract
Oral squamous cell carcinoma (OSCC) is among the most prevalent malignancies worldwide and exhibits considerable variability in clinical outcomes. Current therapeutic strategies often include chemotherapeutic agents; however, their toxicity toward normal tissues is associated with significant adverse effects. Polyphenol extract from Olive Oil is a natural product that demonstrates anticancer activity through multiple mechanisms, including antioxidant, anti-inflammatory, and immunomodulatory actions. This study aimed to investigate the effect of Polyphenol Extract from Olive Oil on an OSCC cell line. The human tongue squamous cell carcinoma cell line (SCC-15) was cultured and allocated into three groups: an untreated control group, a group treated with Polyphenol Extract from Olive Oil, and a group treated with doxorubicin (DOX). In the MTT assay, the extract and DOX were each tested at five logarithmic concentrations (100, 10, 1, 0.1, and 0.01 mg/mL). Cell viability and cytotoxicity (IC50) were evaluated using the MTT assay. We analyzed cell cycle distribution and apoptosis by flow cytometry, and quantified caspase-3 and epidermal growth factor receptor (EGFR) expression levels using enzyme-linked immunosorbent assay (ELISA). Polyphenol extract from Olive Oil demonstrated anticancer activity; however, its efficacy was significantly lower than that of DOX (IC50 = 71.9 ± 4.07 versus 13.4 ± 0.8 mg/mL; $P < 0.001$). Treatment with Polyphenol Extract from Olive Oil decreased cell viability and EGFR expression, while enhancing apoptosis and caspase-3 expression compared with the untreated control group. Cell viability decreased in a concentration-dependent manner across the five tested logarithmic concentrations, whereas apoptosis, cell cycle, caspase-3, and EGFR were assessed at a single concentration of each agent. Polyphenol extract from Olive Oil exhibits a dose-dependent cytotoxic effect against OSCC cells. Although it does not demonstrate sufficient efficacy to replace conventional chemotherapeutic agents, it may serve as a promising adjuvant therapeutic approach.
Keywords. Oral Squamous Cell Carcinoma; Polyphenols; Doxorubicin; Epidermal Growth Factor Receptor.

Introduction

Cancer remains one of the most significant non-communicable diseases affecting public health worldwide. In developed countries, it represents the second leading cause of mortality. Oral squamous cell carcinoma (OSCC) accounts for approximately 96% of all oral malignancies [1-3]. Current treatment modalities for OSCC include surgery, chemotherapy, and radiotherapy. Despite their clinical effectiveness, these approaches are frequently associated with undesirable adverse effects, particularly chemotherapeutic agents, which exert cytotoxic effects on both malignant and normal cells [4]. In recent years, increasing attention has been directed toward the use of natural products as therapeutic or adjunctive agents. Such compounds have been reported to reduce cancer risk while producing fewer adverse effects and promoting overall health [5,6].

Polyphenols constitute the largest group of phytochemicals naturally occurring in plants. Several studies have demonstrated that diets rich in polyphenol-containing fruits, vegetables, and whole grains are associated with a reduced risk of cancer, cardiovascular diseases, and chronic inflammatory conditions [7,8]. Previous investigations have shown that plant-derived Polyphenols may inhibit the initiation of neoplastic transformation or exert antiproliferative effects, thereby reducing the likelihood of cancer development [9]. The anticancer activity of Polyphenol extract from Olive Oil is thought to be largely attributable to the antioxidant properties of its phenolic and Polyphenolic constituents, which efficiently scavenge free radicals and reactive oxygen species (ROS). Additionally, olive-derived compounds such as oleuropein, tyrosol, hydroxytyrosol, and verbascoside have been shown to protect against several forms of cancer [10].

Accordingly, Polyphenol Extract from Olive Oil has been investigated in various malignancies, including human bladder and breast cancer cell lines, where it exhibited cytotoxic and antiproliferative effects [11,12]. Nevertheless, its role in oral cancer, particularly OSCC, remains inadequately explored. Therefore, this study evaluated the anticancer effects of Polyphenol Extract from Olive Oil on OSCC and investigated its potential as an adjunctive preventive and therapeutic strategy to reduce the morbidity and mortality associated with this malignancy.

Materials and Methods

Materials

The polyphenol extract from olive oil was obtained from a private extraction facility and subsequently evaluated at the National Research Centre. The oral squamous cell carcinoma cell line (SCC-15) was purchased from the Confirmatory

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Diagnostic Unit, Research and Development Sector, VACSERA, Cairo, Egypt. The SCC-15 cell line was obtained directly from the Confirmatory Diagnostic Unit, Research and Development Sector, VACSERA (Cairo, Egypt), which performs cell line characterization. Following receipt, the cells were maintained in our laboratory for less than six months; therefore, re-authentication was not considered necessary, as characterization had already been performed by VACSERA before supply.

Study design

The human tongue squamous cell carcinoma cell line (SCC-15) was sub-cultured into three experimental groups: (1) a control group (OSCC cell line without any intervention), (2) a Polyphenol Extract from Olive Oil group, and (3) a DOX group. In the MTT assay, the extract and DOX were each tested at five logarithmic concentrations (Log 2 to Log −2; see below). The flow cytometry (cell cycle and apoptosis) and ELISA (caspase-3 and EGFR) assays compared the three groups, using a single concentration of each agent as described in the corresponding sections.

MTT assay for cellular viability

Before the MTT assay, 100 µL of SCC-15 cells (5 × 10^4 to 1 × 10^5 cells/mL, i.e., 5 × 10^3 to 1 × 10^4 cells per well) was seeded into each well of a 96-well culture plate and incubated for 24 hours in a CO_2 incubator. Following incubation, the culture medium was removed, and cells were washed with phosphate-buffered saline (PBS). Serial concentrations of Polyphenol Extract from Olive Oil (100 mg/mL, 10 mg/mL, 1 mg/mL, 0.1 mg/mL, and 0.01 mg/mL, corresponding to Log 2, Log 1, Log 0, Log −1 and Log −2, respectively, where Log denotes the base-10 logarithm of the concentration in mg/mL) and DOX were independently applied to the cultured cells and incubated for 48 hours at 37°C (treatment period). Cells were washed twice with PBS and continuously monitored using an inverted phase-contrast microscope. Subsequently, culture medium containing 0.5 mg/mL MTT was added, and the cells were incubated for an additional 4 hours at 37°C in a CO_2 incubator. The medium was removed, and the produced formazan crystals were dissolved using dimethyl sulfoxide (DMSO). Optical density was measured at 570 nm using a BioTek FLx800 microplate reader. Cell viability percentages were calculated relative to untreated control wells, and the half-maximal inhibitory concentration (IC50) was determined and is expressed in mg/mL. In summary, cells were allowed to attach for 24 hours, treated for 48 hours, and then incubated with MTT for 4 hours before absorbance was read.

Cell cycle analysis by flow cytometry

Polyphenol extract from Olive Oil and DOX were administered at a single concentration each (the respective IC50 values, 71.9 and 13.4 mg/mL); untreated cells served as the control. Cells were harvested by trypsinization, and the resulting cell suspensions were centrifuged for 15 minutes, fixed for a minimum of one hour at 4°C, and subsequently washed. Cell cycle analysis was performed using a Becton Dickinson (BD) fluorescence-activated cell sorter. At least 20,000 cells were analyzed per sample to determine cell cycle distribution. The percentages of cells in the G0/G1, S and G2/M phases were calculated from the DNA-content histogram and sum to 100% for each group. The Pre-G1 (sub-G1) population, which contains apoptotic cells with fractional DNA content and is not a cell cycle phase, was quantified separately as a percentage of the total events analyzed and is therefore reported in addition to, and not as part of, the 100% distributed among the three phases.

Measurement of apoptosis

Apoptotic activity was evaluated by staining cells with Annexin V and propidium iodide (PI), followed by flow cytometric analysis. This technique utilizes the translocation of phosphatidylserine (PS) from the inner to the outer leaflet of the plasma membrane, a hallmark feature of apoptotic cells. Cells were treated with the same concentrations and for the same duration as for the cell cycle analysis. Early apoptotic (Annexin V+/PI−), late apoptotic (Annexin V+/PI+) and necrotic (Annexin V−/PI+) cells were quantified, and total apoptosis is reported as the sum of these three populations.

Measurement of proliferation (epidermal growth factor receptor)

Following treatment with Polyphenol Extract from Olive Oil and DOX for 24 hours at a single concentration each (the respective IC50 values, 71.9 and 13.4 mg/mL; untreated cells served as the control), cells were harvested, centrifuged, and incubated for 30 minutes at 37°C in a CO_2 incubator. An ELISA-based enzyme assay was then performed to determine the inhibitory effects of polyphenols from Olive Oil and DOX on EGFR protein expression. Caspase-3 was measured by ELISA in samples prepared under the same treatment conditions.

Results

Assessment of cell viability

OSCC-15 cell viability in the intervention groups was assessed using the MTT assay. At all log concentrations (Log 2 = 100, Log 1 = 10, Log 0 = 1, Log −1 = 0.1, and Log −2 = 0.01 mg/mL), the DOX group showed a decrease in viable cell percentage

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compared with Polyphenol Extract from Olive Oil, a highly statistically significant decrease, except at log concentration = -1, where there was no statistically significant difference.

Assessment of cytotoxicity

Using the MTT assay, the higher cytotoxic potential was detected in the DOX group, represented by the lowest IC50 value (13.4 ± 0.8 mg/mL), while the IC50 value of Polyphenol Extract from Olive Oil was 71.9 ± 4.07 mg/mL. The difference was highly statistically significant ($P < 0.001$).

Apoptosis analysis

Regarding total apoptosis, there was a highly statistically significant difference between the Polyphenols Extract from Olive Oil, DOX, and negative control groups ($P < 0.001$). Pairwise comparisons revealed that the DOX group showed the greatest mean total apoptosis percentage, followed by the Polyphenols Extract from Olive Oil group, while the lowest mean was recorded in the negative control group. For early apoptosis, late apoptosis, and necrosis, there was a statistically significant difference among the three groups, but pairwise comparisons revealed no statistically significant difference between the Polyphenols Extract from Olive Oil and DOX groups.

Assessment of caspase-3 expression

Using ELISA, caspase-3 was detected in all studied groups. There was a highly statistically significant difference in caspase-3 concentration among the three groups ($P < 0.001$), with the greatest mean in the DOX group, followed by the Polyphenols Extract from Olive Oil group, while the lowest mean was recorded in the negative control group. Pairwise comparisons revealed that the difference was statistically significant among the groups (Figure 1).

Cell cycle analysis

At the pre-G1 and G2/M phases, the greatest accumulation of OSCC-15 cells was detected in the DOX group, followed by Polyphenols Extract from Olive Oil. At the G0/G1 and S phases, the greatest percentage of cells was observed in untreated OSCC-15 cells, followed by Polyphenols Extract from Olive Oil, while the DOX group showed the lowest value. Overall, the greatest accumulation of DOX-treated OSCC-15 cells was in the G2/M phase, while Polyphenols Extract from Olive Oil and control groups showed the highest accumulation of cells in the G0/G1 phase (Figure 2). In each group, the G0/G1, S and G2/M percentages sum to 100%; pre-G1 (the sub-G1 apoptotic fraction) was measured separately and is reported in addition to these phases, so the four values in Table 1 total 101.9%, 117.6% and 121.6% for the control, extract and DOX groups, respectively.

Table 1. Summary of results

Test	Subgroup	Control	Polyphenols Extract from Olive Oil	DOX
Cell Viability (%)	Log 2 (100 mg/mL)	-	49.7±1.6	40.3±0.4
	Log 1 (10 mg/mL)	-	56.9±0.3	50±1.1
	Log 0 (1 mg/mL)	-	67.9±1	62.4±1.5
	Log -1 (0.1 mg/mL)	-	80.9±4.2	79.3±0.6
	Log -2 (0.01 mg/mL)	-	92.3±1.8	86.6±1.2
Cytotoxicity (IC50, mg/mL)	—	-	71.9±4.07	13.4±0.8
Apoptosis (%)	Total	1.9±0.3	17.6±3.5	21.5±4.4
	Early	1±0.2	5.9±1	8.1±1.5
	Late	0.55±0.1	8.9±1.6	9.7±2.1
	Necrotic	0.35±0.1	2.8±0.7	3.8±0.9
Caspase-3 Expression	—	44.6±11.9	290.8±18.3	431.8±19.1
Cell Cycle Analysis (%)	G0-G1	53.80	43.20	38.20
	S	26.60	24.20	22.60
	G2-M	19.60	32.60	39.20
	Pre-G1 †	1.90	17.60	21.60
EGFR Expression	—	928±487.2	405 ±14.9	290±15.5

Note: Log = base-10 logarithm of the concentration in mg/mL (Log 2 = 100, Log 1 = 10, Log 0 = 1, Log −1 = 0.1, Log −2 = 0.01 mg/mL); cell viability was measured at all five concentrations for the extract and for DOX. IC50 values are expressed in mg/mL. Apoptosis, cell cycle, caspase-3 and EGFR were measured at a single concentration of each agent. † Pre-G1 (sub-G1) is the apoptotic fraction with fractional DNA content, measured separately from the cell cycle phases; G0-G1, S and G2-M sum to 100% in each group, so pre-G1 is additional to this total. Total apoptosis equals early + late apoptosis + necrotic cells.

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Assessment of EGFR expression

The greatest concentration was observed in the control group, followed by Polyphenols Extract from Olive Oil, while the lowest value was in the DOX group, with a highly statistically significant difference (Figure 3).

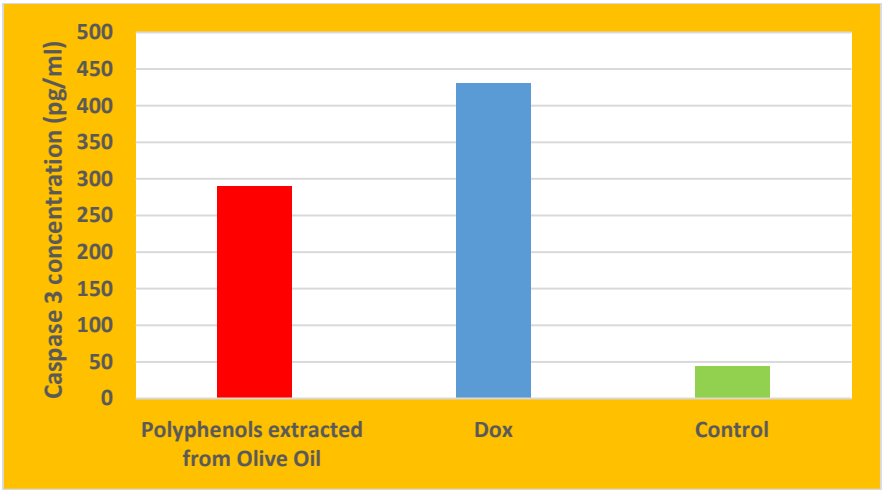

Figure 1. Bar chart representing mean and standard deviation values for caspase-3 concentrations in the three groups.

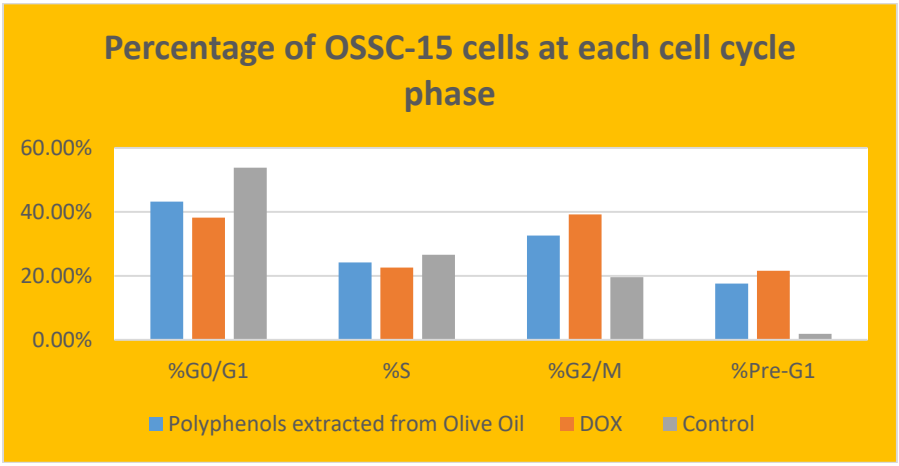

Figure 2. The distribution pattern of cells within various cell cycle phases in OSCC-15 incubated with Polyphenols Extract from Olive Oil and DOX compared with the control group.

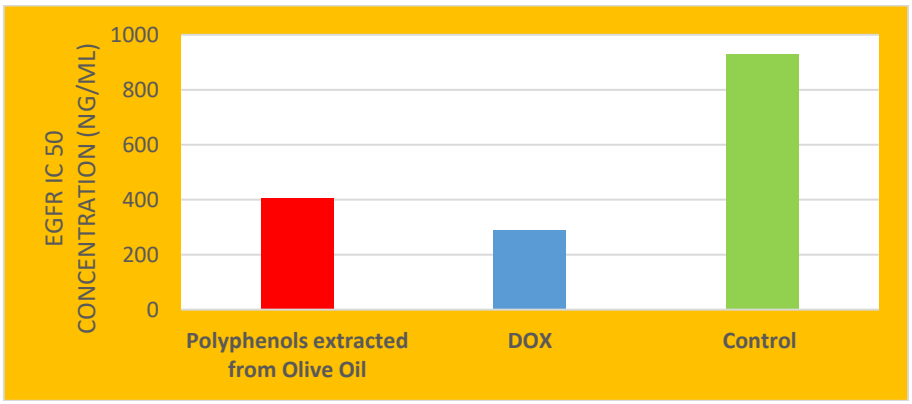

Figure 3. A bar chart illustrating EGFR expression in Polyphenols Extract from Olive Oil and DOX groups compared with the negative control OSCC-15 cell line.

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Discussion

OSCC remains a major global health challenge. Despite substantial progress in diagnostic techniques and therapeutic approaches, survival outcomes remain suboptimal owing to local invasion of critical anatomical structures, treatment-associated toxicity, and the development of resistance to conventional chemotherapeutic agents [13]. Consequently, considerable attention has been directed toward alternative and complementary therapeutic strategies, particularly plant-derived bioactive compounds capable of modulating carcinogenic processes [14]. Polyphenol extract from Olive Oil has recently gained interest because of its potent antioxidant properties [15] and its demonstrated antitumor activity against several human malignancies, including bladder [16] and breast cancers [17].

Given the aggressive biological behavior of tongue OSCC, characterized by early metastatic potential, poor prognosis, and high mortality rates at diagnosis [18], the SCC-15 tongue carcinoma cell line was selected as the experimental model in the present study. The findings of the current investigation demonstrated that both Polyphenols Extract from Olive Oil and DOX reduced SCC-15 cell viability in a concentration-dependent manner. These results indicate that Polyphenols Extract from Olive Oil possesses cytotoxic activity against OSCC cells. This effect may be attributable to the ability of its phenolic constituents to modulate reactive oxygen species (ROS) and cellular oxidative stress pathways, thereby impairing cancer cell survival [21]. Nevertheless, the cytotoxic effect of Polyphenols Extract from Olive Oil remained significantly lower than that of DOX at nearly all tested concentrations. This observation was further supported by IC50 analysis, where DOX exhibited markedly greater cytotoxic potency than Polyphenols Extract from Olive Oil. Given that IC50 reflects the concentration required to inhibit 50% of cellular activity or viability, the substantially lower IC50 value observed with DOX highlights its superior antineoplastic efficacy. Accordingly, Polyphenols Extract from Olive Oil may be considered more suitable as an adjunctive therapeutic agent rather than a direct substitute for conventional chemotherapy. Apoptosis analysis further reinforced the anticancer activity of Polyphenols Extract from Olive Oil. Although DOX induced the highest levels of total, early, and late apoptosis, as well as necrosis, treatment with Polyphenols Extract from Olive Oil resulted in significantly greater apoptotic activity than the untreated control group. These findings suggest that the extract exerts its antitumor effect, at least in part, through activation of programmed cell death pathways. To further clarify the mechanisms underlying apoptosis induction, caspase-3 expression was evaluated.

Caspase-3 is considered a key executioner protease involved in both intrinsic and extrinsic apoptotic pathways. The present study revealed a marked increase in caspase-3 expression following treatment with both DOX and Polyphenol Extract from Olive Oil. Notably, caspase-3 expression in the Polyphenol Extract from Olive Oil group was approximately seven-fold higher than that observed in the negative control group. These findings are consistent with those reported by Elamin et al. [17], who demonstrated that Polyphenol Extract from Olive Oil -induced apoptosis in breast carcinoma cells was associated with caspase-3 activation through mitochondrial-mediated apoptotic pathways. Cell cycle analysis revealed increased accumulation of SCC-15 cells within the pre-G1 (sub-G1 apoptotic fraction) and G2/M phases following treatment with Polyphenols Extract from Olive Oil and DOX. The enrichment of cells at the G2/M checkpoint suggests activation of cell cycle arrest mechanisms that prevent progression into mitosis or interfere with mitotic processes themselves. These findings indicate that inhibition of cell cycle progression may represent an additional mechanism by which Polyphenols Extract from Olive Oil exerts its anticancer activity. Future studies incorporating mitotic index assessment are warranted to determine whether G2 arrest or direct mitotic inhibition is primarily responsible for this effect. These observations are in agreement with the study conducted by Coccia et al. [16], who reported that Polyphenol Extract from Olive Oil induced G2/M cell cycle arrest in urinary bladder carcinoma cells and promoted apoptosis through upregulation of pro-apoptotic proteins and downregulation of anti-apoptotic proteins.

To further investigate the antiproliferative activity of Polyphenol Extract from Olive Oil, EGFR expression was evaluated. EGFR is frequently overexpressed in OSCC and has been associated with unfavourable clinical outcomes and disease progression. In the present study, both intervention groups exhibited significantly reduced EGFR expression compared with the control group. However, the inhibitory effect of DOX was more pronounced than that observed with Polyphenols Extract from Olive Oil. Collectively, the present findings demonstrate that Polyphenol Extract from Olive Oil possesses measurable anticancer activity against OSCC cells through multiple mechanisms, including suppression of cellular proliferation, induction of cell cycle arrest, promotion of apoptosis, activation of caspase-3, and downregulation of EGFR expression. Although these effects were broadly comparable to those elicited by DOX, their magnitude was significantly lower. Therefore, Polyphenol Extract from Olive Oil should be regarded as a promising complementary or adjuvant therapeutic agent rather than a replacement for established chemotherapeutic drugs. Overall, this study provides evidence that Polyphenol Extract from Olive Oil exerts concentration-dependent antiproliferative effects and, at the concentration tested, pro-apoptotic effects against OSCC cells and highlights its potential utility as an adjunctive strategy in the management of oral squamous cell carcinoma.

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Conclusion

Polyphenol extract from Olive Oil exhibits a dose-dependent cytotoxic effect against OSCC cells. Although it does not demonstrate sufficient efficacy to replace conventional chemotherapeutic agents, it may serve as a promising adjuvant therapeutic approach.

Conflict of interest disclosure

The authors declare that they have no conflict of interest.

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