

EFFICACY OF PRUNUS ARMENIACA ON ORAL SQUAMOUS CELL CARCINOMA CELL LINE: AN IN-VITRO ANALYSIS OF A CELL LINE

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ABSTRACT

The Egyptian apricot, or *Prunus Armeniaca*, is a member of the Rosaceae family of plants and has many beneficial compounds, such as amygdalin, polyphenols, fatty acids, and carotenoids. This research aims to assess the anticancer effects of PA extract on OSCC. The OSCC cell line (Scc-25) of human tongue was cultured for 24 hours in DMEM and PA extract was obtained from Giza governorate. Apoptosis, necrosis, cell cycle analysis, mitochondrial membrane potential, caspase-3 and -8 activity, and cytotoxicity tests were conducted to determine the most effective extract. PA induced more apoptosis than staurosporine in OSCC cells, with caspase-3 and -8 levels significantly higher in group-1 than in group-II. Cell cycle analysis showed 7.7-fold higher apoptosis and necrosis in group-1 than in group-2, with an IC50 of 6.83 ug/ml. Apoptosis-inhibiting gene expression has been linked to an increase in cell survival, and novel cancer studies focus on apoptosis dysregulation as the first step in the carcinogenic process. PA has been shown to be less cytotoxic than Straurosporine. Amygdalin in apricot and peach kernel extracts has anti-proliferative effects on human colon cancer cells, suggesting potential as an anti-cancer drug. Apricots have antioxidant, antimicrobial, and antitumor activities, as well as anti-inflammatory and apoptosis effects.

KEYWORDS: OSCC, Rosaceae family, cancer cells, cell cycle

INTRODUCTION

The Egyptian apricot, or *Prunus Armeniaca*, is a member of the Rosaceae family of plants. Its fruit is well-known, and it is widely grown in temperate

climates. Traditional medicine practitioners value it for its high concentrations of beneficial compounds such as amygdalin (cyanogenic glucoside), polyphenols (antioxidants), fatty acids, and carotenoids. (*Fратиanni et al., 2018*).

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PA and its many compounds and byproducts have many useful pharmacological effects. The nourishing role it plays because to its high concentration of nutrients is just one of these benefits (Fратиanni et al., 2018; Cassiem & de Kock, 2019).

2013, Gomaa demonstrated that both sweet and bitter apricot kernels have powerful antibacterial, antioxidant, and anticancer effects. According to her research (Gomaa, 2013), apricots show promise as a potential new medical natural product. Psoriasis treatment was initially thought to be possible because its essential oils have been shown to have substantial antiproliferative effects on cultured human epidermal keratinocytes in vitro (Fратиanni et al., 2018).

Half of all malignancies affecting the head and neck are oral cancers. Nearly 90% of all cases of oral cancer can be attributed to oral squamous cell carcinoma (OSCC), making it the disease's most prevalent form. Late-stage cancer diagnosis carries a particularly grim prognosis, with mortality rates as high as 90% (Vitório et al., 2020). An unfavorable ratio of cell division to cell death. Either an enhanced proliferation rate or a lower apoptosis rate might lead to unchecked tumour growth. In a recent study (Malsy et al., 2019), inhibiting several kinases makes the alkaloid. Staurosporine a potent apoptosis inducing agent. In a recent study (Malsy et al., 2019),

Despite its widespread use as a traditional medicine, not enough research has been done on the anticancer effects of PA. Mahmoudi et al. conducted a study on the anticancer effect on breast cancer in 2019.

There is not enough evidence to support the therapeutic effects of PA, thus this area is still being researched. Therefore, the purpose of this research is to assess PA extract's impact on OSCC (Mahmoudi et al., 2019).

METHODS

At VACSERA laboratories, the OSCC cell line (Scc-25) (ATCC® CRL-1628™) of human tongue was cultivated for 24 hours in DMEM containing 10% FBS and 1% penicillin- streptomycin at 37°C in a humidified 5% CO₂ atmosphere with 10 ug/ml of insulin (Sigma). Adherent cells were detached from 90-mm dishes with trypsin and subsequently planted in 96-well or 6-well plates for use in experiments.

Preparing the PA extract

The PA seeds were obtained in May of 2020 from the Giza governorate in Egypt, cleaned, macerated, and soaked in 90% methanol for 7 days to make alcohol extract. Methanol clear extract was then obtained after the solution was filtered through gauze three times. A dried methanolic extract was suspended in distilled water after the alcoholic extract was filtered and evaporated using a rotary evaporator and freeze dryer (Ningbo Scientz Biotechnology co., LTD, China). The proportion of living cells and the IC₅₀ (the concentration at which half of the cells are killed) were used to determine which extract was the most effective.

Grouping:

Cultured OSCC cell lines were split into two groups.

PA alcohol extract was used on the OSCC human tongue cell line (Group -1).

Group 2 served as a negative control and was comprised of an untreated human tongue OSCC cell line.

Performed tests

Apoptosis, necrosis, cell cycle analysis, and mitochondrial membrane potential ($\Delta\psi_m$) assay were carried out, along with a caspase-3 and -8 activity assay.

Assay for caspase-3 and -8

Expression of caspases 3 and 8 was measured by real-time PCR. In 6-well culture plates, OSCC-25 cells were seeded at a density of 3×10^6 cells/well and incubated as specified. The RNA was isolated and reverse-transcribed with the help of the RNeasy Mini Kit (QIAGEN). Using the BIORAD iScript™ One-Step RT-PCR Kit, we were able to reverse-transcribe and amplify the target RNA. The gene-specific primers used were: Casp 3 F 5'-ctcggctctgtacagatgtcga-3', Casp 3 R 5'-catggctcagaagcacaac-3', Casp 8 F 5'-ACAATGCCAGATTCTCCCTAC-3', and Casp8R5'-CAGACAGTATCCCCGAGGTTT-3'.

All assays were performed in triplicate, and expression levels were determined using the Ct technique. The relative abundance of mRNAs was quantified using the 2-CT method to yield an n-fold change.

Cytotoxicity

Our assay was an MTT-based in vitro toxicology kit. The MTT assay is ideally suited for use with multiwell plates when determining cytotoxicity in vitro. Log phase cells were used, and the ultimate cell density should be kept below 106 cells/cm². The MIC of PA was determined by comparing it to that of the chemotherapeutic drug staurosporine.

Measurement of mitochondrial transmembrane potential ($\Delta\Psi_m$)

Overnight, 1.2×10^4 OSCC-25 cells/well were cultured in a warm medium in a 96-well plate before being treated in the ways specified. After 20 minutes in an incubator (37°C, 5% CO₂), 10 l of 2 mM TMRE labelling solution was added, and the tubes were rinsed three times with warm 1X PBS. BD FacsCalibur was used to measure the fluorescence (excitation 550 nm, emission 580 nm).

Examining data statistically.

Statistical analysis

The results of the $\Delta\phi$ m test were recorded as the mean standard error (n = 3) of Relative fluorescence units (Δ RFU) or relative to RFU of the untreated cells, and the results of the cell viability and cytotoxicity experiment were recorded as the relative optic density of group I compared to group II.

RESULTS

Assay for caspase-3 and -8

Caspase-3 and -8 levels were found to be significantly higher in group-1 than in group-II (2.972 and 2.165 folds, respectively; Figure1-).

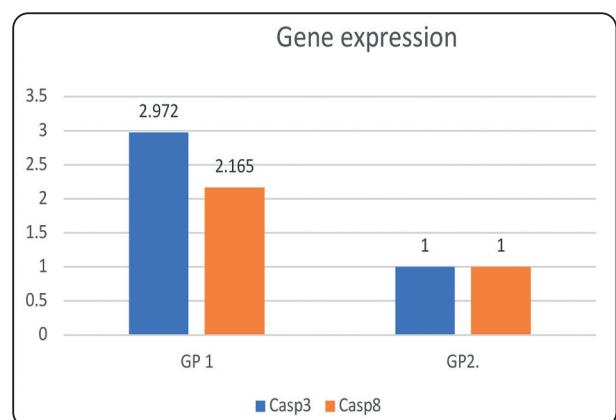


Fig. (1): Showing caspase-3 & -8 assay

Cell cycle analysis

Cell growth arrest at S-phase and overall apoptosis 7.7-fold higher in group-1 than in group-2 was found through cell cycle analysis. (Figure 2).

Apoptosis & Necrosis

The apoptosis rate in OSCC was found to be 7.8 times higher after PA treatment compared to the control group. Similar to the control group, it caused necrosis, albeit at a much lower 4 fold increase. (Table 1, Figure 3).

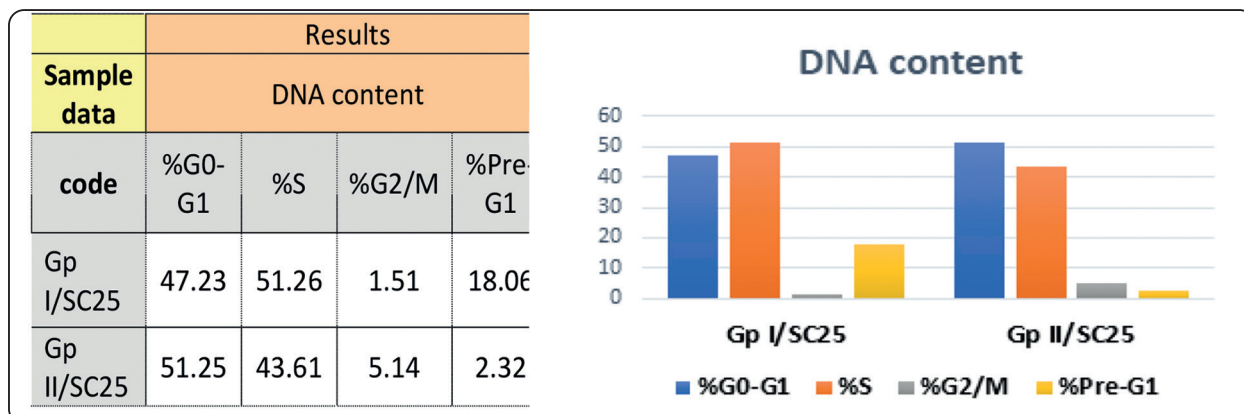


Fig. (2) Bar chart depicting the findings of the cell cycle study for groups I and II.

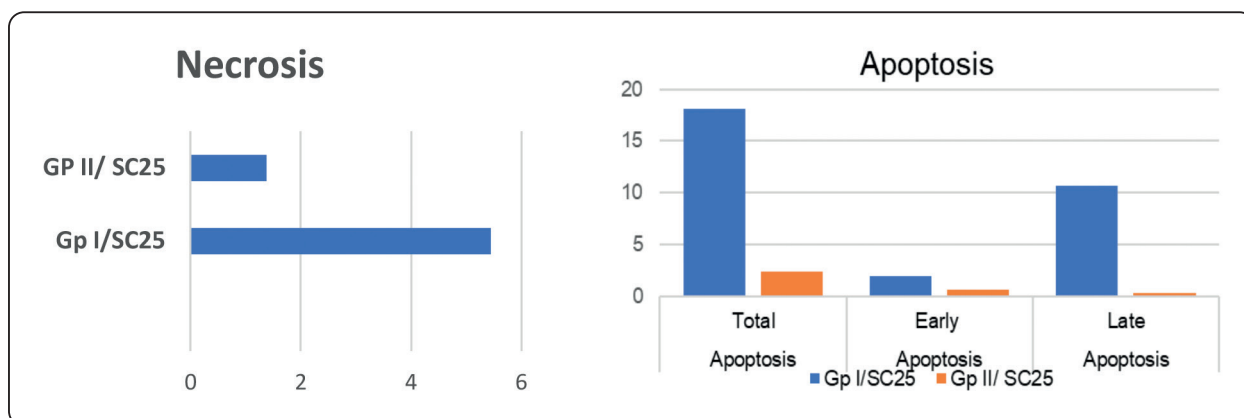


Fig. (3) Represents (a) necrosis in both Groups I and II (b) displays complete apoptosis, including both early and late stages.

TABLE (1): Total apoptosis, as well as early and late apoptosis, can be shown for both groups.

	Apoptosis		
	Total	Early	Late
GPVSC25	18.06	1.91	10.71
.GPIVCont	2.32	0.61	0.33

Cytotoxicity: Drugs as Standards Straurosporine

PA was shown to have low cytotoxicity in comparison to the chemotherapeutic drug Staurosporine, with an IC50 of 6.83 ug/ml compared to 81 ug/ml (Figure 4). The cytotoxicity of Group 1 was significantly lower than that of Staurosporine.

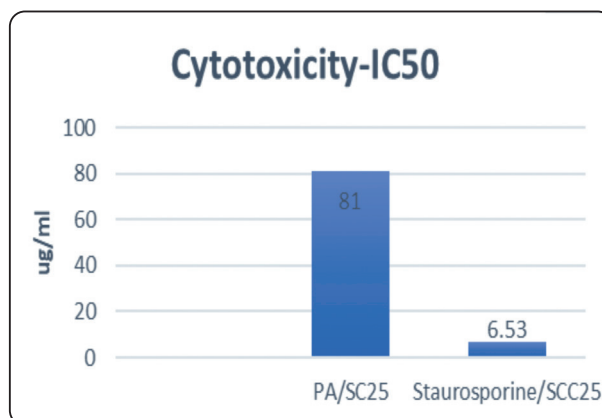


Fig. (4): Cytotoxicity of the PA vs staurosporine

DISCUSSION

In the OSCC cell line, we found that PA induced more apoptosis than the negative OSCC control group. This was evident from assays for caspase 3 and caspase 8, which showed that the extrinsic and intrinsic apoptotic pathways had been activated. Our study found that compared to an untreated OSCC cell line, OSCC cells treated with PA had a 7.8-fold increase in the rate of apoptosis and a growth arrest at the S phase of the cell cycle.

In addition, necrosis was four times as common in the PA group as it was in the control group. Loss of cell viability, which is particularly targeted in cancer cells, was induced by a drop in mitochondrial membrane potential from 66.21% in the untreated group to 33.79% in the PA group. With an IC₅₀ of 81 µg/ml, PA was shown to be less cytotoxic than the chemotherapeutic drug Straurosporine, which was determined to be cytotoxic at a concentration of 6.83 µg/ml. IC₅₀.

When cells undergo development or cellular stress, they may undergo apoptosis, a unique form of cell death in which a cascade of processes occurs, ultimately resulting in the disposal of damaged or unnecessary cells. Apoptosis-inhibiting gene expression has been linked to an increase in cell survival. Therefore, novel cancer studies focus on apoptosis dysregulation as the first step in the carcinogenic process. Several studies have shown that apoptosis plays a crucial part in tumor development (Mahmoudi et al., 2019).

Similar to our findings, *Mahmoudi et al. 2019* investigated how PA affects the expression of the highly upregulated Bax (Bcl-2-associated X) and c-FLIP (Cellular FLICE-inhibitory protein) genes in human breast cancer. The expression of the Bax and c-FLIP genes was greatly reduced by PA. Therefore, they theorized that PA might be connected to the suppression of anti-apoptotic genes in human breast cancer. (Mahmoudi et al., 2019)

Another trial comparing PA to the gold standard treatment of prednisolone was conducted by *Minaiyan et al.* on rats with ulcerative colitis. The PA group was better than the control group, especially when it came to intraperitoneal administration. They concluded that the elevated bioavailability of the active component upon injection was responsible for the observed effect (Minaiyan et al., 2014).

Furthermore, *Cassiem & Kock 2019* observed that human colon cancer cells treated in vitro with South African kernel extract exhibited an intra S-phase halt in the cell cycle. Amygdalin was reported to induce pyknosis or necrosis by decreasing ATP levels. They came to the conclusion that compared to normal cells, human cancer cells had higher quantities of enzymes that cause growth arrest when exposed to amygdalin. According to their findings, these extracts have potential as cancer preventatives. (Kock and Cassiem 2019).

On the contrary, an animal study that disputed our findings concluded that the amygdalin found in apricot seeds posed a risk to the animals' health because it altered the livers' microscopic structure. However, the investigation was unable to confirm the harmful effect at the maximum dose provided (Kolesárová et al., 2020).

Summary and Suggestions

Cell cycle arrest in s-phase was also induced by PA extract, in addition to apoptosis induction via intrinsic and extrinsic routes. This data suggests that PA extract has anti-growth effects on the OSCC cell line derived from the human tongue. The current study is a promising first step in exploring PA's potential as an anti-cancer drug.

Ethical clearance

The FD-BSU Research ethics committee has given its stamp of approval for this study (FDBSUREC/11022021/FA).

• All Authors Agreed to Have Their Work Published

List of Abbreviations:Prunus Armeniaca: **P.A****Oral squamous cell carcinoma :OSCC****REFERENCES**

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