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RESEARCH

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Nutraceutical with a promising oral anticancer effect: in vitro study on apricot oil extract

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Abstract

Background: One of the major threats to human health is malignancy. Treatment regimens usually followed by either chemo or radiotherapy have a wide range of collateral unwanted side effects. Scientists seek alternatives with less or no adverse consequences. Nutraceuticals possess disease-modifying implications in medicine related to Alzheimer's, cardiovascular, Parkinson's diseases and malignancy. Apricot oil extract is one such nutraceutical. Its active component is amygdalin, known also as vitamin B.17 or laetrile. It is found in a wide range of vegetable species. Amygdalin is found in high concentration in the kernels of rosaceous fruits such as bitter nuts and apricot kernels. Amygdalin is an aromatic cyanogenic component with a glycoside group. It has a diverse effect on different systems and organs of the body. Recently, it has shown an anticancer potential as it can decompose carcinogenic elements found in the body and kill malignant cells, so it results in cancer growth inhibition. There is a great debate related to the cyanide toxicity of amygdalin. The presented study aims to evaluate apricot oil extract's impact on squamous cell carcinoma of the tongue, HNO97, while testing its safety on non-tumorigenic oral epithelial cells.

Results: HNO97 cell line viability was markedly decreased. This may be induced by the upregulation of autophagy, apoptosis enhancement as well as cell cycle arrest. Cancer cell migration was also decreased. Apricot oil caused no significant inhibition of normal OEC viability in low doses.

Conclusions: Apricot oil extract from apricot kernel had a notably antitumorigenic impact on oral cancer cells. It may be later subjected to pre- as well as clinical trials.

Keywords: Apricot oil, Amygdalin, Vitamin B17, Laetrile, Tongue carcinoma

Background

Head and neck malignancy is a special entity; one-third of them are oral cancer with 90% of squamous origin. Treatment protocol includes surgery in conjunction with radiotherapy or chemotherapy. In spite of these advances in treatment, patient survival has not shown significant improvement (Attar et al. 2010). This may be attributed to collateral side effects of therapeutic agents that compromise the patient health status. Scientists seek to find novel elements with no or reduced negative impact on different body systems (Omura 2014a).

Medicinal products of natural origin have emerged as an alternative way to treat oral cancer (Weng et al. 2019).

Nutraceuticals are naturally occurring or fortified food supplement that helps not only in treating but also in preventing many diseases. Nutraceuticals can be beneficial in medicine. Nutraceuticals have not been yet assessed and tested clinically as pharmacological agents (Nasri et al. 2014).

Apricot oil extract is one such nutraceutical plant product containing glycosides. The whole oil is extracted from seeds of members of Rosaceae family including Prunus armeniaca (apricot), Prunus amygdalus var. amara (bitter

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almond) and Prunus persica (peach). The main glycoside of this oil is known as amygdalin. Amygdalin is a member of the cyanogenic glycoside family. It contains two glucose molecules, a benzaldehyde molecule and a hydrocyanide (HCN) one. Studies proved that the analgesic effect of amygdalin is attributed to its benzaldehyde component and that the hydrocyanide part is responsible for its antitumorigenic property (Martin et al. 2021).

Fishman et al., in 1940, found that malignant cells extracted from different tissues such as uterus, esophagus, and breast have a higher content of β-glucosidase enzyme compared to normal cells. They stated that increased level of β-glucosidase enzyme is perhaps a characteristic feature of malignant cells. They also stated that cancer cells contain low levels of rhodanese enzyme (Aamazadeh et al. 2020). So, malignant cells have two characteristics, increased amount of β-glucosidase degradable by amygdalin and an insufficient level of rhodanese enzyme with a detoxification power, i.e., which enhances hydrocyanic acid detoxification (Aamazadeh et al. 2020).

When amygdalin is absorbed, malignant cells decompose it and liberate HCN. If extra HCN is leaked from cancerous cells, rhodanese enzymes in surrounding normal cells denature it by converting it into non-toxic thiocyanate. This explains why amygdalin is cancer cell-specific and denies the idea of amygdalin toxicity (Aamazadeh et al. 2020).

Amygdalin can decompose carcinogenic products in the body and block the source of nutrition for malignant cells and so inhibit their growth and kill them (Sireesha et al. 2019).

Laboratory experiments showed that treating cancer cells with amygdalin leads to the upregulation of pro-apoptotic Bax protein and caspase-3 and downregulation of antiapoptotic BcL-2 protein. This enhances the apoptosis of malignant cells. Amygdalin also inhibits the adhesion of breast cancer cells as expression and also by inhibiting the Akt-mTOR pathway, and this will prevent metastases of cancer cells.

This study aims to elucidate the anticarcinogenic effect of apricot oil extract on oral cancer cell line HNO97 derived from tongue squamous cell carcinoma and to reveal its exact molecular mechanism, and at the same time to assess its safety on normal OEC.

Methods

Assessment of apricot oil effect on tongue human squamous cell carcinoma cell line (HNO97 cells)

Culture and propagation of cell line cells

Cells extracted from tongue carcinoma were purchased from the Nawah Scientific Group (Mokattam, Cairo, Egypt). Cells were cultured, expanded and then kept in DMEM media to which 100 mg/mL of streptomycin, 100 units/mL of penicillin and 10% of heat-inactivated fetal bovine serum were added. Culture plates were incubated in 5% CO2 at 37 °C.

Apricot oil extract preparation

Apricot oil obtained from dried apricot seeds contains a great amount of amygdalin. Just before starting the experiment, apricot seeds were prepared to extract the oil. Seeds were washed, dried and then grinded. The supercritical fluid extraction (SFE) technique has been used. This method uses supercritical fluid *carbon dioxide* as the solvent.

Critical conditions of carbon dioxide were = 30.9 °C and 73.8 bar.

Extraction was done at food industry department laboratories at NRC. Sterilization of oil was done by DMSO. The extracted apricot oil was freshly dissolved in cell culture medium (1–10 mg/mL) and applied to tumor cells in culture flasks for 24 h. In all experiments, treated tumor cell cultures were compared to non-treated cultures. Tongue squamous cell carcinoma cell line (HNO97 cell line) was obtained, and cytotoxicity of the cells was observed based on the morphological changes.

IC 50 calculation

The viability of malignant cells after adding apricot oil extract was assessed using SRB assay. Aliquots containing 100 µL of suspended cells (5×10^3 cells) were put in 96-well plates and then were incubated in DMEM for 24 h. After that, cultured cells were treated with apricot oil extract at various concentrations. After 72 h, cell media was discarded and we added 150 µL of 10% TCA and incubated at 4 °C for 1 h to fix cells. Then, TCA solution was removed, and cells were washed adequately with distilled water. SRB solution (0.4%w/v) were added and plates were incubated for 10 min in dark. Plates were then washed adequately by acetic acid (1%) and kept to air dry overnight. The absorbance was measured then at 540 nm using a microplate reader (Ortenberg, Germany) (Fig. 1).

The percent cell viability was calculated using the following formula:

$$\%\text{Viability} = \text{O.D of Control sample} - \text{O.D of Test sample} / \text{O.D of Control Sample.}$$

Recording morphology of HNO97 cells under light microscope

To assess morphological changes of cells in different culture media, cells were seeded in 60-mm dishes at a density of 5 × 105 cells/mL. Then, we added calculated

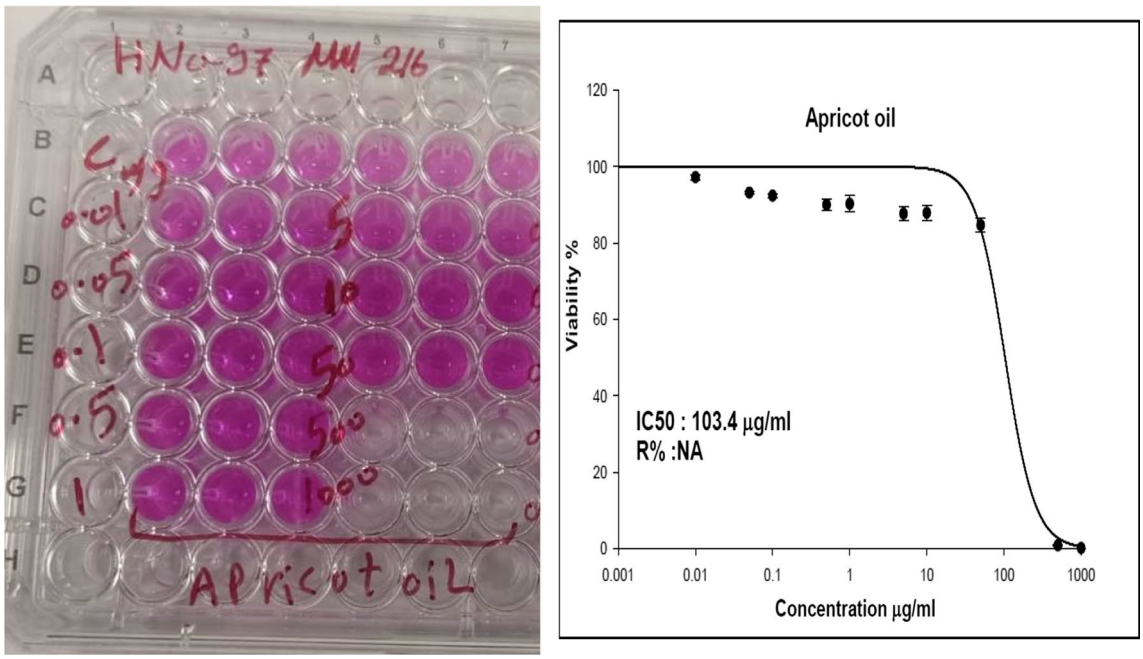

Fig. 1 SBR assay to calculate cytotoxicity of apricot oil extract and determine IC50

apricot oil extract IC50 (105 µg/mL) for 72 h. We then examined and recorded cell morphology under an inverted light microscope (Olympus, Germany).

Analysis of DNA content of tumor cells based on cell cycle assessment

DNA % in cells was assessed by analyzing the cell cycle and its phases using flow cytometry. After treatment with apricot oil extract IC50 (105 µg/mL) for 72 h, cells (105 cells), we collected tumor cells by trypsinization. We washed cells twice with iced PBS (pH 7.4). We then resuspended cells in 2 mL 60% iced ethanol, and we incubated cells at 4 °C for 1 h to fix them. In a tube containing 1 mL of PBS containing 50 µg/mL RNAase A and 10 µg/mL propidium iodide (PI), cells were resuspended. After that, nuclei were isolated and stained them with PI. According to the instructions of the manufacturer, we analyzed on FACSCalibur flow cytometer. Each cell cycle phase was analyzed for DNA content using ModFit software (ModFit, Topsham, ME, UK).

Assessment of autophagy and formation of autophagosome by acridine orange stain

During autophagy, proteins in the cytoplasm are sequestered and acidic vesicular organelles are arising (AVO). To quantitatively assess autophagy, we stained cells with acridine orange stain and the results are evaluated by flow cytometry. After treatment with apricot oil extract IC50 (105 µg/mL) for 72 h, cells (105 cells), we collected tumor cells by trypsinization. We washed cells twice with iced PBS (pH 7.4). Cells were then stained with acridine orange (10 µM) and incubated in dark at 37 °C for 30 min. To detect the presence of acidic vesicular organelles (AVO) (Weng et al. 2019), a flow cytometer (BD FACSCanto II) with red (650 nm, stained by cytoplasmic vesicles) versus green (510–530 nm, stained nuclei) fluorescence (FL3/FL1) from cells illuminated with blue (488 nm) excitation light were measured. A minimum of 10,000 cells within the gated region were analyzed. The intensity of fluorescence was proportional to two factors: the volume of the intracellular acidic compartment as well as its volume.

Flow cytometric analysis of apoptosis

Annexin-V FITC fluorochromes apoptosis detection kit was used to assess apoptosis and necrosis of cultured cells (Abcam Inc., Cambridge Science Park, Cambridge, UK). After treatment with apricot oil extract IC50 (105 µg/mL) for 72 h, cells (105 cells), we collected tumor cells by trypsinization. We washed cells twice with iced PBS (pH 7.4). Cells were transferred to a tube with the addition of 5 µL of FITC-conjugated annexin-V (annexin-V FITC) and 5 µL of PI (PI 50 mg/mL). After that, cells were incubated for 15 min at room temperature. Dilution of stained cells was by the binding buffer and then cells were analyzed by flow cytometer (BD FACSCalibur). Four separate populations of cells can be distinguished using this technique: unlabeled population (viable cells), the population bound to only

annexin-V FITC (early apoptotic), the population stained with PI (necrotic), and those stained with annexin-V FITC and also labeled with PI (late apoptotic). The distribution of fluorescent results was presented as a color dot plot analysis, and percentage of fluorescent cells in each quadrant was determined. Apoptotic cells were double Annexin-V FITC/P-positive cells.

Assessment of tumor cell migration/wound healing assay

To evaluate the power of tumor cells to migrate and assess their motility potential, wound healing assay was used. Ti was chosen for its simplicity and high reproducibility. This is a non-expensive method to study cancer cell migration in vitro. This test relies on developing an artificial wound in the form of a scratch and observing if the cells are able to re-establish contact between them.

After plating cells at a density of 3×105/well onto a coated 6-well plate, they were cultured overnight in routine culture medium at 37 °C and 5% CO2. After reaching confluency, scratches were made horizontally into the confluent monolayer. Then, we washed the plates carefully with PBS. Control wells were covered with 5 mL of routine culture medium, and cells to be tested were treated with media to which apricot oil extract IC50 (105 µg/mL) was added. Images were taken using an inverted light microscope at 0, 24, 48 and 72 h. Taken photographs were displayed below and analyzed by MII ImageView software version 3.7.

Assessment of cytotoxic effect on normal oral epithelial cell line

Cell culture

OEC: Oral epithelial cell was obtained from the Nawah Scientific Company (Mokattam, Cairo, Egypt). Cells were cultured in routine culture media supplemented with 100 mg/mL of streptomycin, 100 units/mL of penicillin and 10% of heat-inactivated FBS in 5% CO2 at 37 °C.

OEC viability assay

The viability of OEC after adding apricot oil extract was assessed using SRB assay. Aliquots containing 100 µL of suspended cells (5×10^3 cells) were put in 96-well plates and then were incubated in DMEM for 24 h. After that, cultured cells were treated with apricot oil extract at various concentrations. After 72 h, cell media was discarded and we added 150 µL of 10% TCA and incubated at 4 °C for 1 h to fix cells. Then, TCA solution was removed, and cells were washed adequately with distilled water. SRB solution (0.4%w/v) was added, and the plates were incubated for 10 min in dark. The plates were then washed adequately with acetic acid (1%) and kept to air-dry overnight. The absorbance was measured at 540 nm using a microplate reader (Ortenberg, Germany) (Fig. 2).

Fig. 2 Cytotoxicity assay of apricot oil extract on normal OEC using SRB assay

The percent cell viability was calculated using the following formula:

$$\%\text{Viability} = \text{O.D of Control sample} - \text{O.D of Test sample.} / \text{OD of Control Sample.}$$

Statistical analysis

Collected data were tabulated and analyzed using SPSS16® (Statistical Package for Scientific Studies), GraphPad Prism and Windows Excel. Data were presented as minimum, maximum, mean and standard deviation. Different groups were compared using one-way ANOVA test and then by Tukey's post hoc test for multiple comparisons. The significance level (P value) was insignificant as P value > 0.05 which indicated data originated from normal distribution (parametric data) resembling normal bell curve.

Results

Effect of apricot oil extract on tongue squamous cell carcinoma cell line (HNO97):

Cell morphology

In apricot oil extract-treated group, morphological alterations were observed (Figs. 3, 4). Cells are detached and become floating in the culture plates. These morphological changes were characteristic of apoptotic cells (dead cells). Apricot oil extract inhibited the proliferation of HNO97 cells as they did not reach confluency as did the control cells (Fig. 5). Cell density was clearly decreased compared to control. These changes

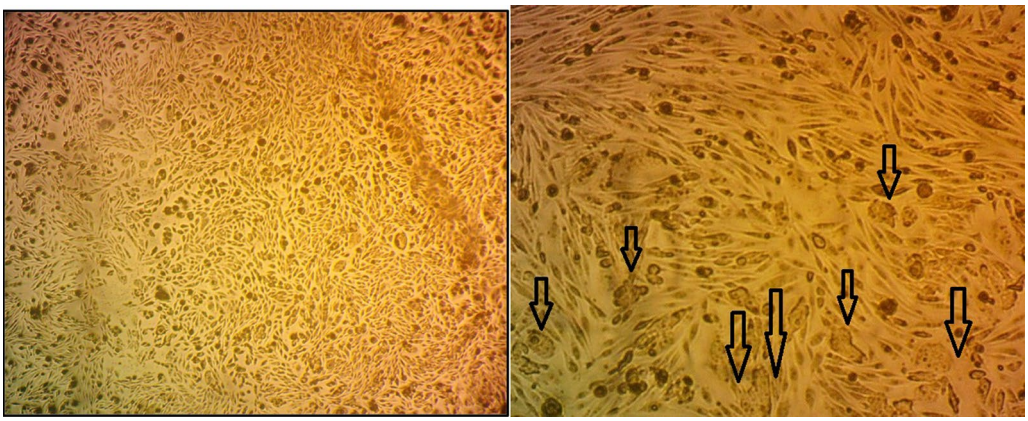

Fig. 3 Photomicrograph of HNO97 cells cultured in 105 µg/mL apricot oil (×40, ×200)

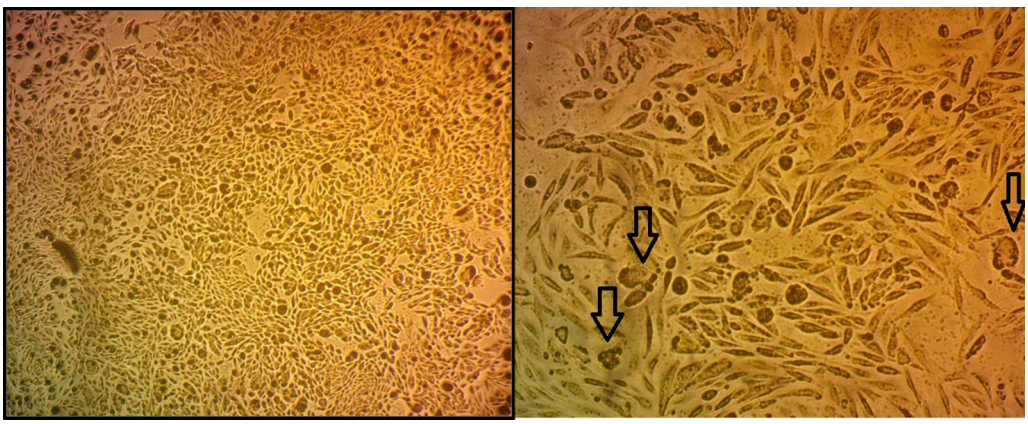

Fig. 4 Photomicrograph of HNO97 cells cultured in 200 µg/mL apricot oil (×40, ×200)

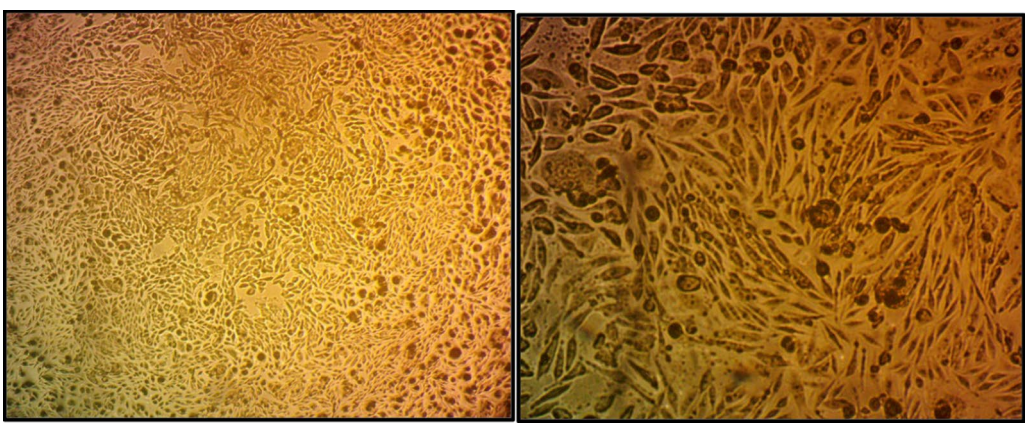

Fig. 5 Photomicrograph of control HNO97 cells cultured in routine culture media (×40, ×200)

are dose-dependent, i.e., the more the concentration, the less is the cell confluency, indicating the death of cells as confluency is a measure of cell viability.

Annexin-V for Apoptosis assessment:

When apricot oil extract is added to culture media, apoptotic percentage increases compared to control

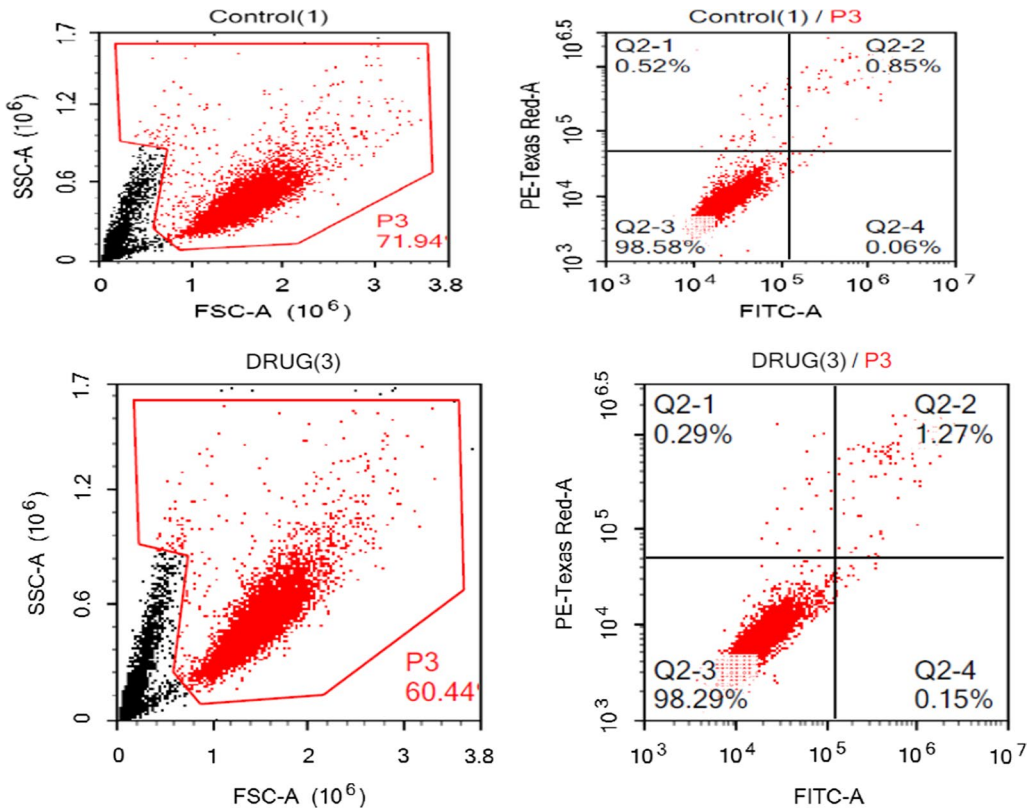

Fig. 6 Flow cytometry for apoptosis analysis of cultured HNO97 cells treated with apricot oil extract

cells. Cells treated with apricot oil extract showed an increase in both Q2 and Q4 cell fraction compared to control untreated cells. *Early apoptosis phase (Q4)*: Cells in this phase still have intact cell membranes (early phase apoptotic cells) *Late apoptosis phase (Q2)*: Cells in this phase have damaged cell membranes filled with pores (late phase apoptotic cells) (Fig. 6).

Flow cytometric analysis of cycle of cells

Close analysis of the cycle of HNO97 cells cultured with and without apricot oil revealed that cells were arrested in G1 phase. This was evident by an increase in cell percentage in this phase from 57.78% (control) to 67.55% (treated cells). This is accompanied by a decrease in the percentage of cells in S as well as G2 phase (22.09% and 13% control to 17.78% and 9.95% treated) (Fig. 7).

Autophagy

Acridine orange stain was used to confirm autophagy in cultured cells. Being a lyotropic stain, it depends on pH to accumulate in organelles with a lower pH. When the pH increases till the organelle becomes neutral, acridine orange becomes green. When the pH drops, this stain is trapped in the organelles after being protonated and accumulates with the emission of red bright fluorescence. As shown in Fig. 8, flow cytometric analysis after acridine orange staining showed an increase in red fluorescence intensity after drug treatment indicating enhancement of AVOs (Fig. 8). Histogram profiles in Fig. 8B show the mean fluorescence intensity of control and drug-treated cells. Our current research showed an increase in red fluorescent structures in HNO97 cells after apricot oil treatment for 72 h compared to control (Fig. 8), which was the evidence of AVO formation.

Wound healing assay/cell migration assay

The experiment was done in triplicates; control images are denoted as C, and drug images are denoted as D. Wound width can be calculated as the average distance between the edges of the scratches. The wound width decreases as cell migration is induced. Results are displayed as mean ± standard deviation (Table 1, Figs. 9, 10).

Assessment of cytotoxic effect on normal oral epithelial cell line

A comparison between all groups was made. It was obvious that there is a significant difference between them ($P < 0.05$). Tukey's post hoc test was achieved to

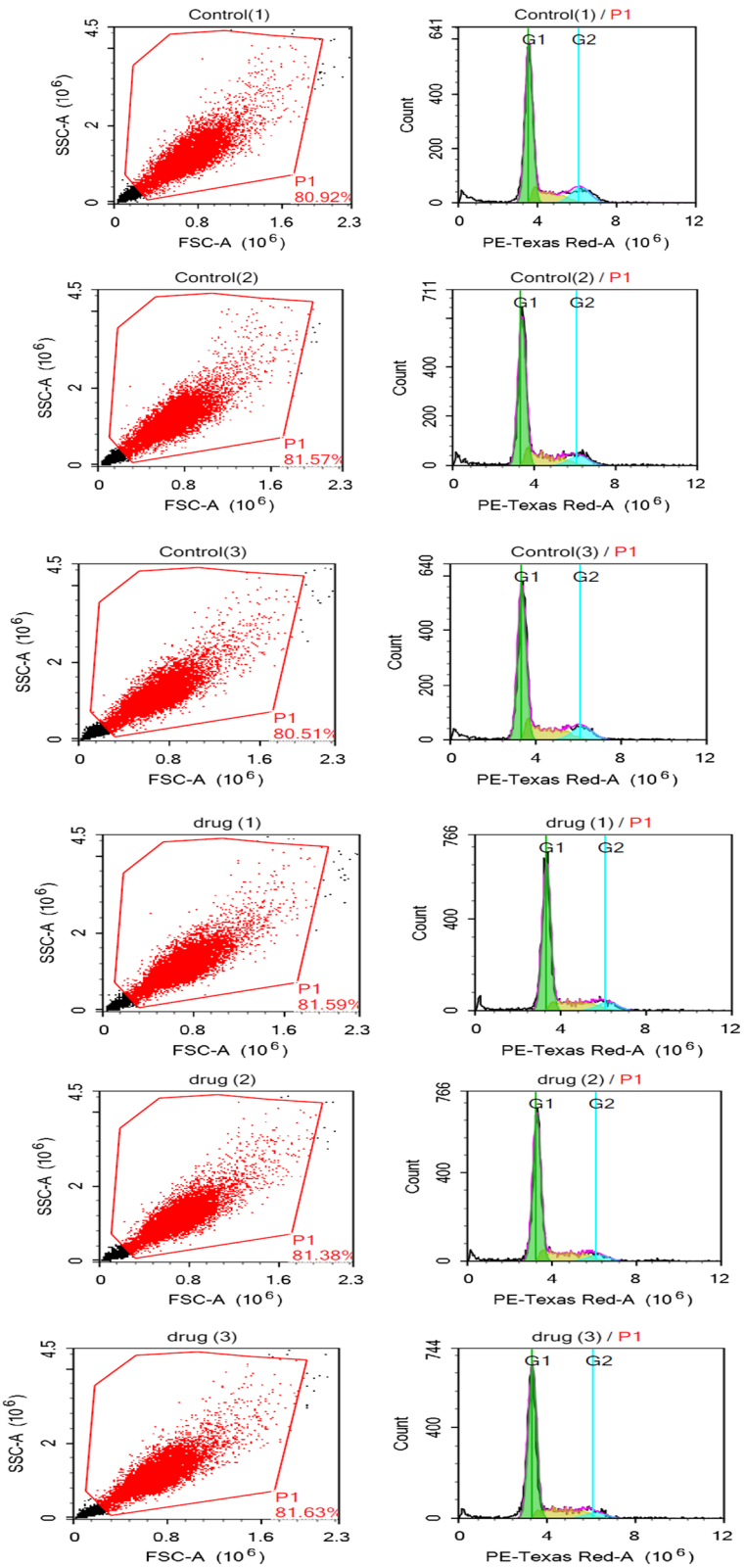

Fig. 7 Flow cytometry for cell cycle analysis of cultured HNO97 cells treated with apricot oil extract

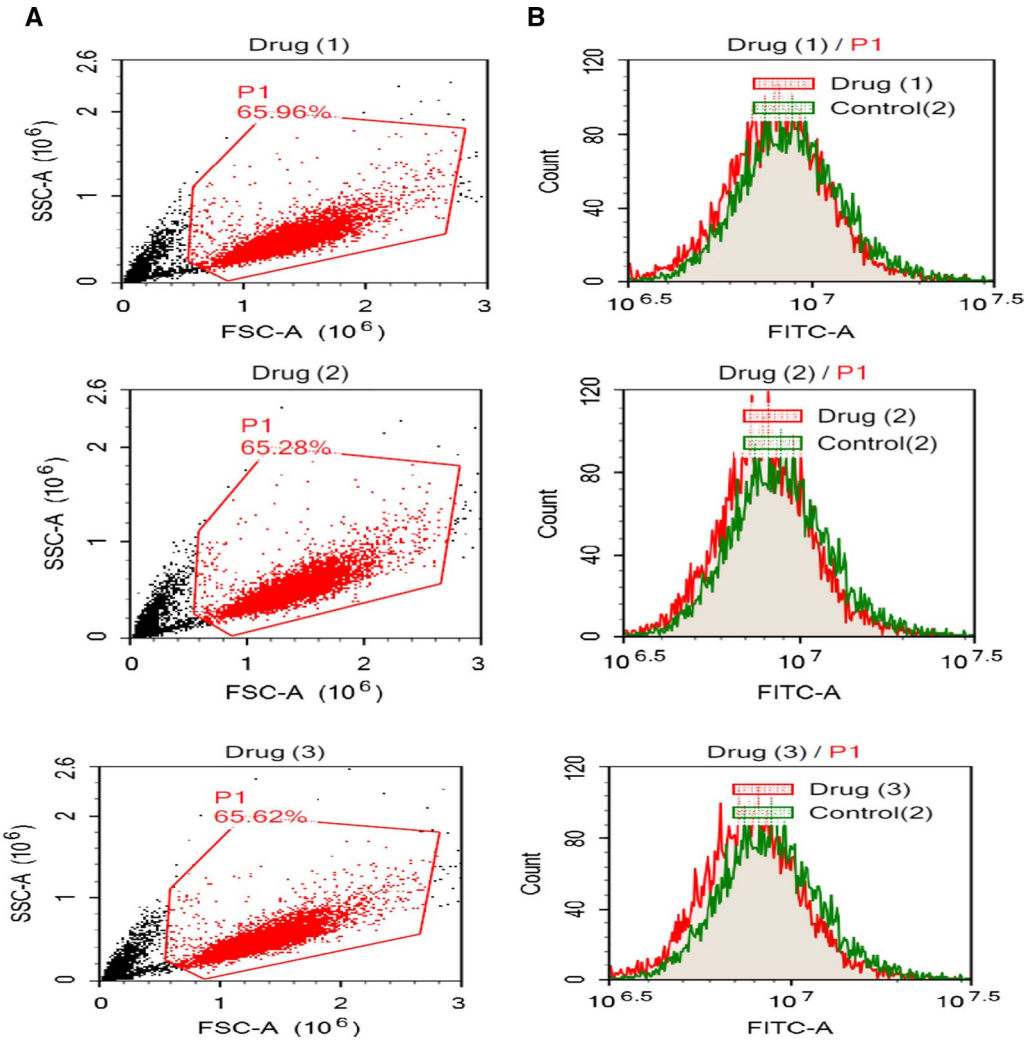

Fig. 8 **A** Flow cytometric analysis after AO staining. **B** Histogram profiles of the control and apricot oil group were generated by flow cytometry

Table 1 Wound width in control and apricot oil extract group in different groups

	Control						Drug				
	C1	C2	C3	Average	SD		D1	D2	D3	Average	SD
0 h	4.3	4.25	4.2	4.25	0.05	0 h	4.25	4.39	4.27	4.30333	0.07572
24 h	2.05	2.19	1.01	1.75	0.64467	24 h	3.16	2.82	2.88	2.95333	0.18148
48 h	2	2.12	0.91	1.67667	0.66666	48 h	3.08	1.41	2.62	2.37	0.86261
72 h	0	0	0	0	0	72 h	1.66	0	2.02	1.22667	1.07747

compare between many groups. Apricot oil extract with a dose of (200) was significantly the lowest as $P < 0.05$, while there was an insignificant difference between all others as $P > 0.05$, as presented in Table 2 and Fig. 11.

Discussion

Although the challenges facing clinicians aiming to optimize the management modalities of cancer, oral cancer still constitutes one-third of malignancy of the head and

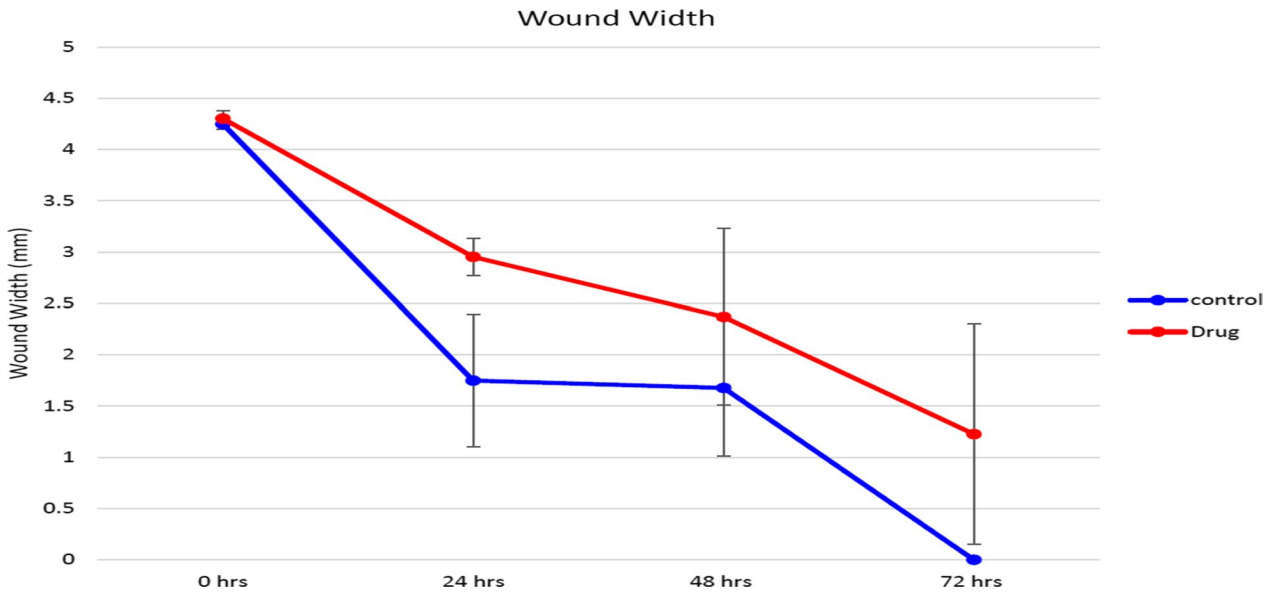

Fig. 9 Graph showing wound width at 0, 24, 48 and 72 h between control group and group of HNO97 cells treated with apricot oil extract. A clear inhibition of cell migration in the treated group is noted

neck region with an increasing curve of morbidity and a declining chance for total cure. Unfortunately, there is no improvement in oral cancer patient survival rate having an average of 5 years. This is not the only burden, as the patient quality of life is still worse. This may explain why researchers and scientists are in a hurry for introducing alternative treatment modalities that may be complementary or substitutive for the conventional ones (Omura 2014b).

For these reasons, green or natural medicine has been introduced in the past years as non-synthetic compounds show little, usually tolerable side effects. Unfortunately, these new agents are still mysterious for clinicians and little is known about their efficacy as well as their exact mechanism of action (Shin et al. 2014).

In this study, we used the oil extract of apricot kernel and we assessed its potentially suggested anticarcinogenic effect on tongue carcinoma cell line with reference to its cytotoxicity on normal non-tumorigenic oral epithelial cell line. As new drugs and pharmacological agents must be widely studied before introducing them to clinicians for clinical studies, cell lines show validity and reliability for studying and discovering these agents in labs (Sarin et al. 2020).

Although used since the 1940s, apricot oil extract, containing a large amount of amygdalin, still represents a great debate concerning its cytotoxicity on normal non-cancerous tissues and organs.

In the present study, different concentrations of apricot oil extract were used (0–300 µg/mL) and a dose–response curve was plotted from which IC50 was calculated and was ~105 µg/mL. Apricot oil extract in its IC 50 was able to inhibit cancer cell line growth and decrease cell survival in HNO97 cell line in a dose-dependent way. There was an insignificant decrease in cell viability of normal OEC cultured in a media supplemented with different concentrations of apricot oil extract. Only at a high dose of 200 µg/mL, a significant inhibition of cell growth started to show.

This may be attributed to the fact of presence of β-glucosidase enzyme in cancer cells and its absence in normal cells. At the same time, normal cells have rhodanese enzyme that is absent in malignant cells. β-glucosidase enzyme breaks down amygdalin after its absorption and liberates HCN which is toxic to malignant cells. If some of this HCN leaks out to surrounding normal cells, rhodanese enzyme is responsible for its detoxification to non-toxic thiocyanate. The combinatorial balance between these two enzymes is responsible for the cyanide cytotoxicity related to malignant cells while escaping normal cells (Aamazadeh et al. 2022).

The research conducted by Sireesha et al. (2019) similarly proved an antiproliferative impact of apricot oil extract on KB oral cancer cell line in a concentration of 100 µgl/mL. In a different way, Makarevic et al. used purified amygdalin instead of the whole oil. They stated that amygdalin inhibited the proliferation of three well-known bladder cancer cell lines in a dose-dependent

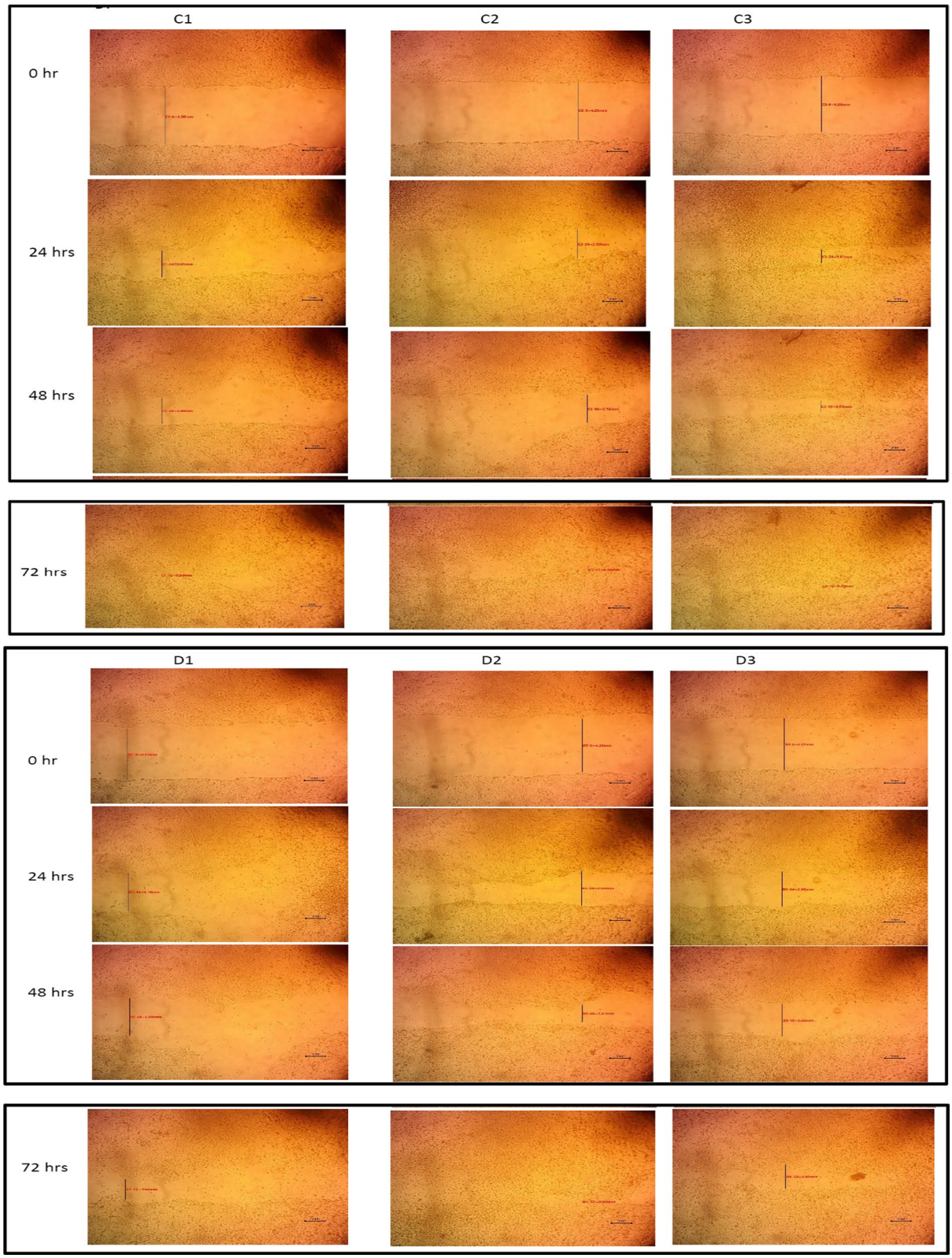

Fig. 10 HNO97 cells migration. D Photographs at different time points: 0, 24, 48 and 72 h following wounding of HNO97 cells treated with 105 µg/mL apricot oil extract. C Photographs at different time points: 0, 24, 48 and 72 h following wounding of untreated HNO97 cells. In each group of cells, six fields were evaluated. Data are presented as mean ± SEM

Table 2 Comparison between the effect of different concentrations of apricot oil extract on OEC

Group	*M*	SD	*P* value
C	1.655[a]	0.022	< 0.0001*
10	1.606[a]	0.023	
20	1.605[a]	0.033	
100	1.603[a]	0.019	
105	1.604[a]	0.005	
200	1.518[b]	0.010	

$P \leq 0.05$ is significant

Concentrations having the same superscript letters have insignificant difference in viability of cells: $P > 0.05$

Concentrations having the same superscript letters have significant difference in viability of cells: $P < 0.05$

* Highly significant

manner and that this was mediated by a cell cycle arrest at G0/G1 phase (Makarević et al. 2014).

Glycosides of plant origin are of two groups: flavonoids and polyphenolic. They have dual action according to their concentration. They are therapeutic in low doses and are toxic in high doses. They are large compounds which are more complex than the mammalian glycosides. This made them have a narrow therapeutic index. This was proved in the present study as an increasing dose of apricot oil extract has made it cytotoxic to normal OEC (Song and Xu 2014).

Similarly, Casseim et al. assessed the effect of organic extract of both apricot and peach kernels. They demonstrated that they affected cell cycle progression and that this was reflected by a reduction in cell viability and proliferation as well as morphological changes of cells indicating cell death as detachment, clumping and nuclear fragmentation (Cassiem and Kock 2019).

Dynamic properties of cells are important to be studied. This means cell cycle phases and DNA content, specific organelle changes as autophagy as well as apoptotic cascades. All the above was made easier by the use of flow cytometry (Nour et al. 2016).

In the present work, cell cycle distribution was evaluated as cell cycle regulation is very crucial for carcinogenesis. There was an increase in G1/S phase DNA content after treatment of HNO97 cells with apricot oil extract denoting cell cycle arrest at this phase. Similarly, Park et al. showed that treating SNU-C4 human colon cancer cells with amygdalin resulted in deregulation of genes involved in cell cycle (Makarević et al. 2016).

Autophagy is a recycling phenomenon for cellular components. It may be induced by many conditions as starvation or as a reaction to added pharmacological agents. When autophagy is induced, there is increased sequestration of non- or dysfunctional organelles with concomitant autophagosomes formation. The latter fuse with lysosomes forming autophagolysosomes which are then degraded via a signaling pathway (Christodoulou et al. 2022). Treating HNO97 cells in this study with apricot oil extract induced autophagy enhanced apoptosis. Chang et al. stated that apoptosis-inducing cell death was enhanced in DU145 and LNCaP human prostate cancer cell line when amygdalin was added to their microenvironment. Apoptotic proteins such as caspase-3 were upregulated, while BCL-2 antiapoptotic protein was downregulated (Aamazadeh et al. 2020). Similarly, Chen et al. (2013) observed that there was a caspase-mediated apoptosis in HeLa cells induced by amygdalin.

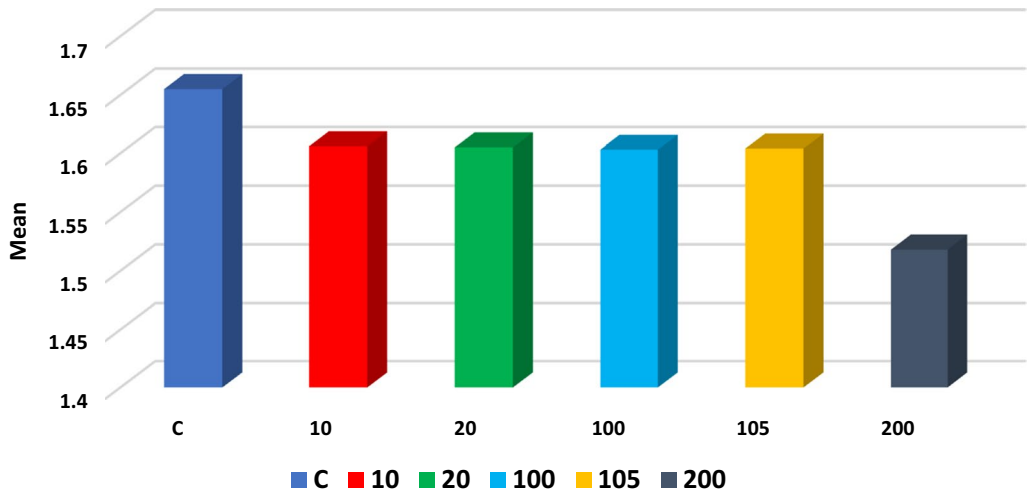

Fig. 11 Comparison between viability of OEC cultured in different concentrations of apricot oil extract

This study analyzed the apricot oil extract effect on cell motility and migration of HNO97 cells. It was able to inhibit cell migration and this was proved by a wound healing assay. Qian et al. (2015) stated an inhibition of in vitro carcinogenic profile of non-small cell lung cancer cells when amygdalin was used. This profile included cell proliferation, as well as migration and also invasion. This was attributed to integrin and E-cadherin regulation as well as AKt-m TOR signaling pathway.

Conclusions

On the basis of these results added to the literature review, more detailed studies and further investigations are mandatory to state apricot oil extract as a novel agent for oral cancer prevention and treatment. Controlled clinical trials are required to further substantiate the anticancer property of this agent on oral cancer. Apricot oil extract successfully inhibited cell line extracted from SCC of tongue via inhibition of viability, cell cycle arrest, apoptosis and autophagy enhancement, and migration inhibition with no detectable cytotoxicity on normal oral epithelial cells.

Malignant cells have β-glucosidase and lack enzyme rhodanese that is capable of converting cyanide to non-toxic thiocyanate. This makes cancer cells more vulnerable to the effect of amygdalin than do healthy non-tumorigenic cells.

Recommendations

Animal models must be studied prior to preclinical studies on human volunteers. This helps in determining the precise dose of apricot oil extract. Similarly, the most effective route of administration must be stated. These studies will allow measuring the plasma level of bioactive ingredients of apricot oil extract after local application on mucosa. This will help reveal any side effects that may develop with prolonged use.

Abbreviations

DMSO: Dimethyl sulfoxide; DMEM: Dulbecco's modified Eagle medium; DMBA: 7,12-Dimethylbenz[*a*]anthracene; SRB: Sulforhodamine B; NRC: National Research Centre; WHO: World Health Organization; AVO: Acidic vesicular organelles; OEC: Oral epithelial cell; ANOVA: Analysis of variance; OD: Optical density; CO2: Carbon dioxide; H & E: Hematoxylin and eosin; MMP-9: Matrix metalloproteinase-9; PI: Propidium iodide; FITC: Fluorescein isothiocyanate; MREC: Medical Research Ethics Committee; OSCC: Oral squamous cell carcinoma.

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Author contributions

MME and HET conceived the study. MME planned the experiment design and was responsible for the in vitro work. HST and HET shared the statistical analysis of the results. Both the manuscript draft and the final one were written, revised, and approved by all authors. All authors read and approved the final manuscript.

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Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article.

Declarations

Ethics approval and consent to participate

Approval was obtained from the Medical Research Ethics Committee of National Research Centre Number (MREC-NRC): 1439102021. The procedures used in this study adhere to the standards of the Declaration of NRC.

Consent for publication

Not applicable.

Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

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