Apricot oil extract as a topical chemopreventive agent in induced tongue squamous cell carcinoma

Heba E. Tarek, Heba N. Shalash, Marwa M. Ellithy

Department of Basic Dental Science, Oral and Dental Research Institute, National Research Centre

Correspondence to Heba E. Tarek, BSc, MSc, PhD, Department of Basic Dental Science, Oral and Dental Research Institute, National Research Centre, 33 El Buhouth Street, Dokki, Cairo 12622, Egypt. Tel: +201222580730; fax: +20233387803; e-mail: hebatarek81@hotmail.com

Received: 15 September 2022
Revised: 20 October 2022
Accepted: 27 October 2022
Published: 23 May 2023

Journal of The Arab Society for Medical Research 2023, 18:26–34

Background/aim
The tongue squamous cell carcinoma (TSCC) is considered one of the primary causes of death worldwide. Despite the presence of different therapeutic modalities, scientists always search for natural products that have an anticancer effect. Apricot oil extract has anticancerous effect, but its use is limited due to its toxic systemic effect. In this study, we aimed to investigate the anticancer effect of apricot oil extract locally applied on induced TSCC.

Material and methods
Thirty rats were divided into three groups, group I was the control group, group II received 7, 12 Dimethylbenz[a]anthracene (DMBA) topically and group III received apricot oil extract alternative with Dimethylbenz[a]anthracene topically. After killing the rats, tongues were dissected and were prepared for histological and immunohistochemical assessment.

Results
Group II developed TSCC with features of malignancy while group III showed hyperplastic epithelium which was dysplastic and started invasion in few cases. Immunohistochemically, group II showed a strong positive expression of Ki-67 and matrix mettaloprotinase 9 compared with group III. Statistical analysis showed that group II was significantly the highest in Ki-67 and matrix mettaloprotinase 9 expression.

Conclusions
From the results obtained, we can conclude that apricot oil has potent anticarcinogenic effect and that using it topically may be an alternative to systemic use.

Keywords:
amygdalin, apricot oil, rats, tongue squamous cell carcinoma

J Arab Soc Med Res 18:26–34

1687-4293

Introduction

Each year 275 000 new cases of oral cancer arise worldwide [1]. Smoking tobacco and alcohol consumption form the main predisposing factors for cancer development [2]. The elementary treatment usually ends with both high morbidity and loss of organ function or 50% recurrence of the tumor which may lead to death [3].

The current treatment of oral cancer involves surgery, chemotherapy, radiotherapy, or sometimes a combination of these modalities, but still there is not considerable advance in survival [4]. For that cause, there is an ongoing advancement in research for new alternative modalities. In spite of this recent advancement, the prognosis of oral cancer is still tacky. Different therapeutic modalities such as surgery, chemotherapy, radiotherapy, and the incoming therapeutic modality gene therapy have been inspected in animal studies. The induction of oral squamous cell carcinoma (OSCC) has been studied in various models, and the carcinogenic agent 7, 12-dimethylbenz(a)anthracene (DMBA) has been confirmed to be used as an effective inducing agent [5].

New therapeutic modalities can be studied *in vitro* and *in vivo* The obstacle to the laboratory studies is the disparity between physiological processes and the cells in culture leading to deceptive results [6]. Mice, rats, and hamsters have been used as models for induced OSCC. Once the induction of OSCC has been confirmed, the tumors are convenient for investigating different treatments in addition to gene therapy [7].

Plant extracts have been introduced as alternatives to the traditional therapeutic medicines. Apricot oil extract is one of the edible plant extracts with a

functional component known as amygdalin or laetrile. The pharmacological properties of Apricot oil extract are thought to be due to the presence of amygdalin. In the United States nearly in 1920s, the first administration of apricot oil was reported. However, it was abandoned at that time due to its toxicity [8]. In 1970s, amygdalin become one of the most prevalent, nontraditional anticancer therapies and 70 000 cancer patients in United States utilized it by 1978 [9]. After that, a Mexican company predestined oral and injectable formulations of amygdalin and found that they did not fulfil the pharmaceutical standards in the United States [10]. Then a prohibition was put on the utilization of amygdalin in the United States. But it remains allowable in 23 states of the United States only for terminally ill cancer patients [9]. National Cancer Institute streaked to analyze its competence as a result of the controversies regarding the use of amygdalin.

Under the approval of American Food and Drug Administration, the National Cancer Institutemade a clinical trial, which failed to prove the efficiency of amygdalin as an antineoplastic agent [9]. There is insufficient information about the toxicity of the natural amygdalin, although it is used as an anticancer agent by many cancer patients [11]. Until now, there is no available data on the local effect of apricot oil extract on OSCC, so the present study aimed to investigate the effect of using apricot oil extract locally on induced tongue squamous cell carcinoma in rats.

Material and methods

Animals

In all, 30 male albino rats (6 weeks old), weighting 120–150 g were obtained from an animal house of the National Research Centre, Cairo, Egypt. They were housed in plastic cages, at the experimental animal unit in the National Research Centre.

Ethical approval

This study was conducted with the Code of Ethics of the World Medical Association, according to the principles expressed in the Declaration of Helsinki. The experiment followed the guidelines for the care and use of experimental animals adopted by Medical Research Ethics Committee of the National Research Centre with approval number 1439102021.

Materials

(1) 7, 12-DMBA was purchased from Sigma-Aldrich (D3254) and dissolved in paraffin oil according to the manufacturer's instructions.

(2) Apricot oil was extracted from apricot kernel by a specialist in the Department of Food Industry, National Research Centre, Cairo, Egypt.

Apricot oil extraction

The apricot kernels were obtained from the apricot fruit, then it was washed and allowed to dry completely. Cracking of the dried kernel was done using a hammer and grinding of the cracked kernel was done before extraction. Then oil extraction was done using supercritical fluid extraction (SFE-CO_2).

Study design

Animals were divided into three groups (10 each) as follows:

Group I (control group): the rats' tongues were painted with paraffin 3 times per week for 8 weeks.
Group II (positive control group): the rats' tongues were painted with DMBA for 3 times per week for 8 weeks.
Group III (apricot oil treated group): the rats' tongues were painted with apricot oil 3 times per week alternatively with DMBA painting for 3 times per week in alternative days for 8 weeks.

Sampling and clinical evaluation

After 8 weeks from starting cancer induction and applying the apricot oil extract or DMBA, anaesthetization of animals by ether inhalation was done, followed by decapitation. The tongue of each animal was examined for any clinicopathological changes. Samples were obtained from the suspected tongue lesions for histopathological evaluation. In addition, clinical evaluation for each rats' tongues were inspected for any changes at 4, 6, and 8 weeks after starting the experiment.

Histopathological evaluation

After the rats were killed, the tissue samples obtained from all groups were fixed in 10% buffered formalin and then paraffin embedded. Sections of 4 μm thickness were mounted on glass slides and stained with hematoxylin and eosin examination under a light microscope to confirm the induced dysplasia or cancer.

Immunohistochemical evaluation

Sections of 4 μm thickness were mounted on positively charged glass slides (OptiPlus, BioGenex Laboratory, Fremont, USA). Then dewaxing of the sections followed by labelling with the selected markers (anti-KI-67 antibody, rabbit monoclonal with

catalog number SAB5500134, and anti-matrix mettaloprotinase 9 (MMP-9) antibody, catalytic domain with catalog number AB19016) were obtained from Sigma-Aldrich. Both were ready to use using an automated autostainer (Thermo Scientific, Lab Vision Corporation TM, Fremont, USA). The immunoreactions were visualized using the UltraView Universal DAB (diaminobenzidine) Quanto Detection Kit in an automated autostainer (Thermo Scientific, Lab Vision Corporation TM, Fremont, USA). All the immunohistochemically stained slides were examined under a light microscope (Olympus System Microscope Model BX43, Tokyo, Japan), coupled to a digital camera (AdvanCam-U3II; Advan Vision Corp., Fukuoka, Japan) for image acquisition. In addition, assessment of area percentage of positive staining was measured by an image analyzer computer system.

Statistical analysis

All the collected data were statistically analyzed using SPSS 16 (Statistical Package for Scientific Studies, Munich, Germany), GraphPad prism and Windows Excel. All data were presented as minimum, maximum, mean, and SD of KI-67 and MMP-9 markers in all groups. Reconnaissance of the collected data was completed using Shapiro–Wilk test and Kolmogorov–Smirnov test for normality, which revealed that the significant level (*P* value) was insignificant as *P* value greater than 0.05 which indicated data originated from normal distribution (parametric data) resembling a normal bell curve. Accordingly, comparison between different groups was performed by using one-way analysis of variance (ANOVA) test followed by Tukey's post-hoc test for multiple comparisons. Also, correlations between both markers in all groups were performed by using Pearson's correlation coefficient.

Results

Clinical evaluation

Regarding the endurance of the rats to the chemical carcinogenic agent (DMBA), rats of group III endured well the oral exposure to DMBA with the same mortality rate as group I (control group) while group II expressed a higher incidence of death.

The rats' tongues were inspected for any changes during painting of the tongues, and it was shown clinically that the tongues of group I were normal with normal pink color and normal morphology and were free of any pathological lesions during the whole period of the experiment. For group II, there were variable presentations ranging from mild inflammatory redness of the tongue to severe inflammation from week 2 to week 8 after the induction. Atrophy of the tongue papilla started to take place from 4–6 weeks after induction. Ulceration starts to appear with increase in the severity and ulcer depth from week 6 to week 8. Ulcers were surrounded by severe inflammation. Generally, the frequency of such ulcers gradually increases over time. The number of lesions in group II dramatically increased between week 6 and week 8. For group III, a similar picture was observed as group II but with decrease in the incidence of all features observed in group II. Some cases show exophytic papillary growth, which was observed on the dorsal surface of the tongue of group II as shown in Fig. 1.

Histopathological evaluation

The examined sections in group I show normal epithelium with intact basement membrane (BM) and normal tongue papilla as shown in Fig. 2a. While in group II hyperplastic epithelium with invasion of tumor cells and formation of tumor nests in the subepithelial tissue was detected in almost all cases as shown in Fig. 2b–e. Features of malignancy such as hyperchromatism, pleomorphism, and abnormal mitotic figures were detected. In group III, hyperplastic epithelium with intact BM was shown in most cases. Also dysplastic epithelium with starting of invasion was detected as shown in Fig. 2c, f; moreover, two rats showed no pathological changes in the lining epithelium.

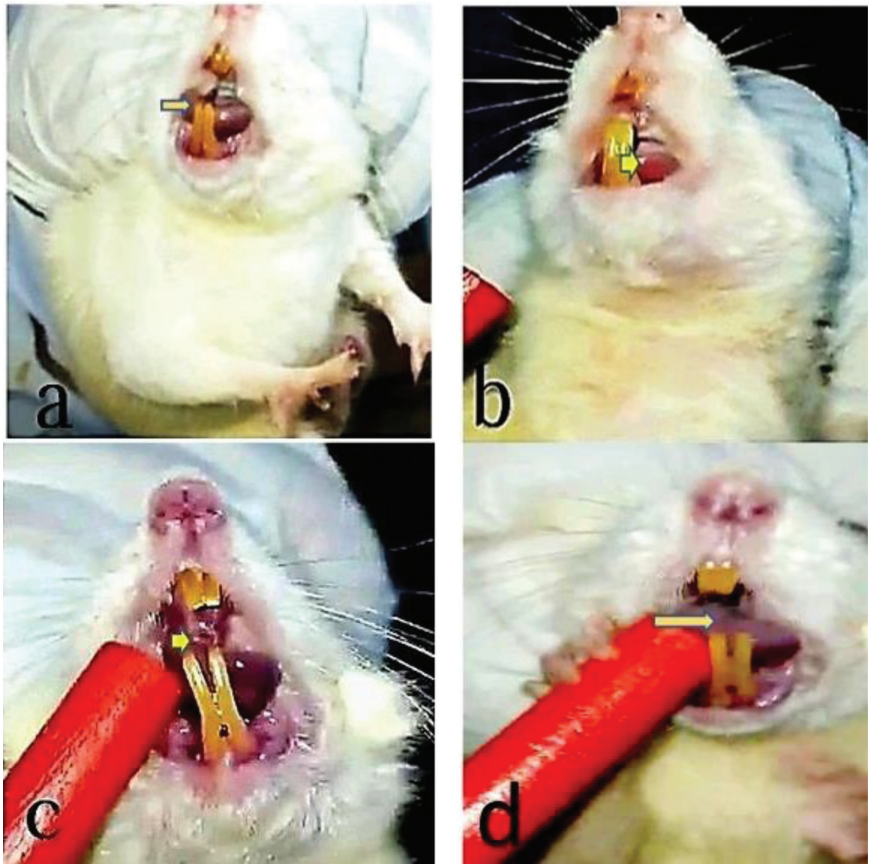
Immunohistochemical evaluation

Ki-67 expression

The immunohistochemical expression of Ki-67 in the rat tongue is shown in Fig. 3, where all tissue sections of group I showed negative immune expression for Ki-67 in the epithelium as shown in Fig. 3a. In group II, there was a strong positive expression of Ki-67 in the epithelium as shown in Fig. 3b, while in group III there was moderate positive immune expression of Ki-67 in the epithelium as shown in Fig. 3c.

In addition, on comparison, the expression of Ki-67 between different groups was performed using one-way ANOVA test which showed significant difference between them as *P* less than 0.05, followed by Tukey's post-hoc test for multiple comparisons, which revealed that group II (18.69±5.09) was significantly the highest group, followed by group III (5.44±2.06), while group I significantly revealed the lowest results (1.18±0.31) as shown in Table 1.

Figure 1



Photograph showing the clinical appearance of the rats' tongues (arrows): (a) severe inflammation of group II tongue at 6 weeks, (b) mild inflammation in group III tongue at 6 weeks, (c) severe inflammation and papillary lesion in group II tongue at 8 weeks, (d) moderate inflammation in group III tongues at 8 weeks.

Matrix mettaloprotinas 9 expression

The immunohistochemical expression of MMP-9 in the rat tongue is shown in Fig. 4, where all tissue sections of group I showed negative immune expression for MMP-9 in the epithelium and stroma as shown in Fig. 4a. In group II, there was positive expression of MMP-9 in the stroma with areas of intense positive expression as shown in Fig. 4b, d, e, while in group III there was weak positive immune expression of MMP-9 in the stroma as shown in Fig. 4c, f.

In addition, on comparison, the expression of MMP-9 between different groups revealed significant difference between them at P less than 0.05, using ANOVA test, followed by Tukey's post-hoc test for multiple comparisons, which revealed that group II (5.23 ±0.80) was significantly the highest group, followed by group III (1.88±1.10), while group I significantly revealed the lowest results (0.60±0.39) as shown in Table 1.

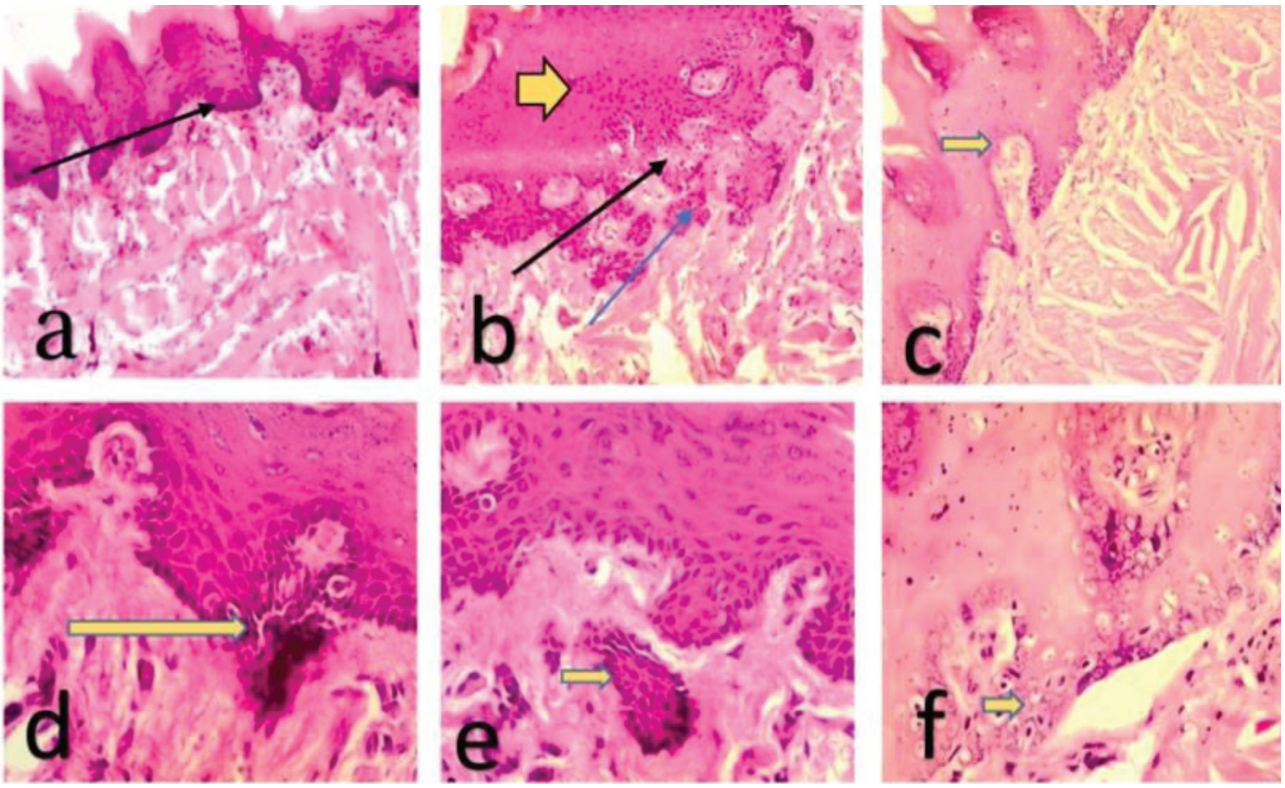
Correlation between KI-67 and MMP-9 markers

Pearson's correlation between both markers was performed and revealed (moderate, positive – insignificant correlation) in group I, while revealed weak, positive – insignificant correlation) in both groups II and III as shown in Table 2.

Discussion

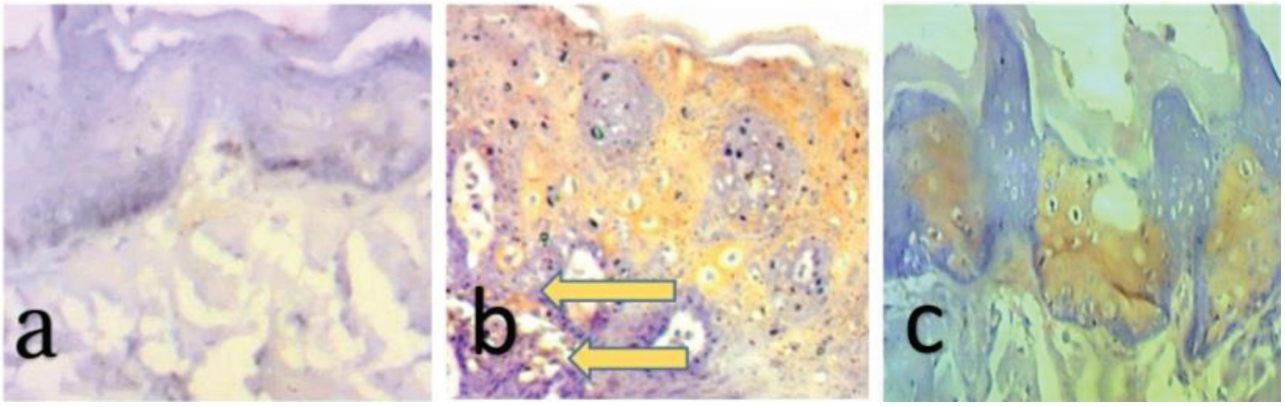
OSCC comprises ~90% of all types of oral cancers. It arises from the surface epithelium; in some studies OSCC and oral cancers are used interchangeably [12]. There is a terrifying increase in the development of OSCC in patients under 45 years old. It is one of the most aggressive tumors, and most of the patients present with advanced stage of the disease at diagnosis, which makes the multimodality protocol of treatment a must. The primary causes of death include lymph node metastasis, invasion of the tumor, high rates of recurrence as well as the evolution of a second tumor. Even if the tumor is diagnosed in the early

Figure 2



Photomicrograph of the rat tongue: (a) group I shows the normal epithelium with intact basement membrane; (b) group II shows hyperplastic epithelium (yellow arrow) with invasion of tumor cells (black arrow) and formation of tumor nests in the subepithelial tissue (blue arrow); (c) group III shows hyperplastic epithelium with intact basement membrane. (H&E, original magnification ×10); (d) group II shows breakdown of basement membrane by malignant cells; (e) group II shows disruption of basement membrane with formation of tumor nests in the stroma, (f) group III shows dysplastic epithelium with starting of invasion (H&E, original magnification ×20). H&E, hematoxylin and eosin.

Figure 3



Photomicrograph of the rat tongue: (a) group I shows negative expression of Ki-67 in the epithelium and subepithelium; (b) group II shows positive Ki-67 immunohistochemical expression in the covering epithelium and basal cells invades the subepithelial layer; (c) group III shows positive Ki-67 immunohistochemical expression in the epithelium (Ki-67, original magnification ×10).

stage, OSCC of the tongue and floor of the mouth are very aggressive as they have increased potentiality for invasion and metastasis. Scientists are trying to understand the biological cascade for cancer initiation as well as its evolution and they try to correlate this with biomarkers which may help in diagnosing oral cancer and can also predict the prognosis of the tumor [13].

For OSCC, the prioritized treatment option is surgery which may require the use of adjuvant radiotherapy or chemotherapy in advanced stages. Recently, targeted

therapy and immunotherapy show auspicious results. Response to treatment is variable between patients and this may lead to cost increase with or without increase of treatment duration, and this may lead to worsening of patient state as many undesirable side effects may develop. Therefore, the scientific community of oral cancer is challenged to overcome these problems [12]. So, there is an insistent need for testing the efficiency of drugs and radiation before their delivery on cancer patients.

Table 1 Immunohistochemical expression of KI-67 and matrix mettaloprotinas 9 in all studied groups

	Minimum	Maximum	Mean	SD
KI-67				
Group I	0.82	1.68	1.18[a]	0.31
Group II	10.69	25.32	18.69[b]	5.09
Group III	2.45	7.78	5.44[c]	2.06
MMP-9				
Group I	0.09	1.29	0.60[a]	0.39
Group II	3.96	6.54	5.23[b]	0.80
Group III	0.53	3.79	1.88[c]	1.10

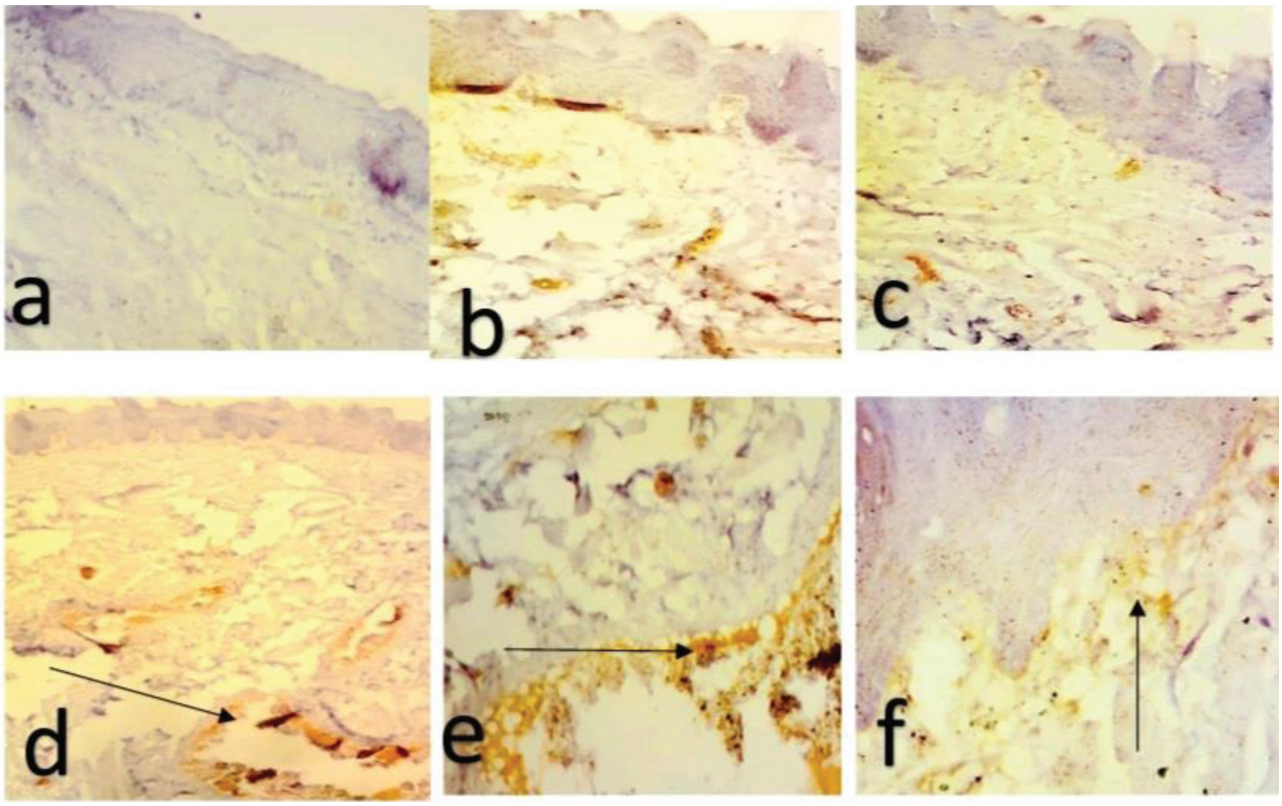
MMP-9, matrix mettaloprotinas 9. [a, b, c]Significantly different at *P* less than equal to 0.05 using analysis of variance test.

As known with other types of tumors, OSCC new drugs either chemopreventive or chemotherapeutic must be first assessed in vitro followed by an in vivo evaluation. Laboratory animals help mimicking human OSCC when chemically inducing these tumors. Using animal models or cell line in vitro are the most frequently used procedures in testing new therapies [12]. Using animal models in preclinical studies allow testing novel drugs before their use on human beings [13,14]. Rarity of oral cancer in animals have guided scientists to develop many models of chemically induced oral cancer in animals to identify biomarkers, investigate risk factors, and find the

Table 2 Pearson's correlation between KI-67 and matrix mettaloprotinas 9 expressions in all groups

	r	*P* value	Indication
Group I	0.51	0.12	Moderate, positive – insignificant correlation
Group II	0.18	0.60	Weak, positive – insignificant correlation
Group III	0.16	0.65	Weak, positive – insignificant correlation

Figure 4



Photomicrograph of the rat tongue: (a) group I showing negative expression of MMP-9 in the epithelium and stroma, (b) group II showing positive expression of MMP9 in the stroma; (c) group III showing positive MMP-9 immunohistochemical expression in the stroma (MMP-9, original magnification ×10); (d) group II showing positive expression of MMP-9 in the stroma (MMP-9, original magnification ×4); (e) group II showing intense positive expression of MMP-9 in the connective tissue, (f) group III showing positive immunohistochemical expression of MMP-9 in the subepithelial layer (MMP-9, original magnification ×20). MMP-9, matrix mettaloprotinas 9

effect of different treatments whether preventive or curative [15].

In this study, we induced tongue squamous cell carcinoma in a rat model by painting DMBA on the rat tongue for 8 weeks in a group and the other group receive extracted apricot oil alternative with DMBA. We aimed to investigate the role of the oil extracted from the apricot kernel to be used in preventing OSCC induction by DMBA. Clinically our results showed that the rats of group III tolerated the DMBA painting more than group II, and this was evident by their good health state as well as their viability compared with group II. Histopathologically, group III showed hyperplastic and in some cases dysplastic epithelium without invasion except in two cases only where no pathological changes could be detected while group II showed dysplastic epithelium with invasion of the stroma in all cases.

Our results are in accordance with a study which investigated the effect of apricot oil on SCC cell line. They found that it caused a significant decrease in viability of cancer cells. And they concluded that the antineoplastic effect of apricot oil is mainly due to the presence of amygdalin, which has an antiproliferative effect caused by enhancement of apoptosis in the cells, so they recommended its use in animal experiments and later in clinical trials [11].

Amygdalin was examined on colorectal and liver cancer cell lines, and found to exert significant cytotoxic effect on cancer cells. And they recommend further studies to detect the safe dose level of amygdalin, which is a natural anticancer agent that is extracted from botanical sources [16].

Saleem *et al*. [17] conducted a review article about the role of amygdalin extracted from apricot kernel on cancer, and they concluded that amygdalin have anticancer activity by causing apoptosis and cell cycle arrest.

Malignant cells have high uncontrollable proliferative capability; the proliferation of malignant cells can be monitored by measuring biomarkers such as Ki-67, which is an important proliferative marker. In our study, we confirmed the induction of tongue SCC by assessing the invasion and proliferation ability of the cells, and these features are important in the carcinogenic profile of OSCC.

In our study by immunohistochemical evaluation of Ki-67, immunostaining showed weak to moderate staining in group III compared with group II indicating a well-marked reduction of tumor proliferation. Our results were confirmed by statistical analysis, the expression of Ki-67 were significantly higher in group II compared with group III indicating less proliferative ability of the lesions treated with apricot oil extract. Nour *et al*. [18] found that amygdalin induces apoptosis of malignant cells in OSCC-induced buccal pouch in hamsters by decreasing both P53 expression and mitotic index rate assessed by Ki-67 expression. There is significant increase of Ki-67 expression in lesions with moderate and poorly differentiated OSCC as well as moderate and severe oral dysplastic epithelium; therefore, Ki-67 can determine the severity of dysplasia and histological grading of carcinoma [19]. Blocking Ki-67 inhibited cell cycle progression [20]. And it was found that Ki-67 is expressed during different cell cycle phases M, S, G1, and G2 but it was not detected in G0 [21]. Expression of Ki-67 was significantly higher in tumor cells than normal cells [22]. Its expression can also predict the recurrence of tumor [23]. In the oral epithelium, Ki-67 expression was restricted in the basal cell layer [24] while in the dysplastic epithelium, it was found to be expressed in basal, parabasal, and spinous layers [25]. And this was in accordance with our findings.

MMPs effectively breakdown the extracellular matrix (ECM) components and BM [26]. In OSCC, the increased expression of MMP-9 is usually associated with an increased metastatic potential of the tumor as well as a decrease in survival rate due to degradation of type IV collagen, which is a main component in the BM. It is also correlated with the aggressiveness of tumor [27]. In our study, we have chosen to evaluate the invasive ability of cancer cells treated by the apricot oil extract to confirm the chemopreventive ability of the oil. By immunohistochemical evaluation of MMP-9, immunostaining showed weak MMP-9 expression limited to the subepithlial zone in group III, while in group II a strong expression of MMP-9 which was extended to different depths in the stroma and this confirms the anticarcinogenic effect of apricot oil extract by decreasing invasive ability of the cancer cells as seen in group III.

Different studies have investigated the use of MMP-9 in detecting the invasive ability of cancer cells. MMP-9 and MMP-2 were assessed in the head and neck SCC, and there were increased expression of MMP-9 in cases with poor prognosis in OSCC, oropharyngeal, laryngeal, and nasopharyngeal cancer. Also, MMP-9

expression was linked with the invasion depth in most tumors of the head and neck; it acts also as an indicator for recurrence in oral cancer [28].

MMP-9 expression was evaluated in the adenocarcinoma of the esophagogastric junction and was found to be associated with invasion of cancer cells and metastasis of the tumor. The author suggested the cause to be due to ability of MMP-9 to degrade type IV collagen in the BM which allows tumor cell invasion [29].

In this study, we investigated the correlation between KI-67 and MMP-9 expression and found positive correlation between the expression of both markers, and this indicates that the cancer cells that have more proliferation ability also have powerful invasion ability. And as the proliferation ability denoted by Ki-67 expression increases, the ability of invasion denoted by MMP-9 expression also increases. MMP family especially MMP-9 is involved in the degradation of ECM and found that it was involved with tumor invasion and metastasis. The ECM remodeling is associated with MMP-9 expression in breast carcinoma, and this increases tumor progression. MMP-9 showed a positive expression in the stroma in breast cancer. They found significant correlation of MMP-9 expression with the expression of cytokeratin 17, Ki-67, CD44, and epidermal growth factor. And MMP-9 significantly increased with increased proliferation of the cells denoted by high Ki-67 expression, and this is linked to poor prognosis [30] and this was in accordance with our results.A study investigated the expression of MMP-2 and MMP-9 in tongue cancer and found a higher expression of the markers than in normal mucosa and dysplastic mucosa. Also, the markers were associated with lymph node involvement. They concluded that their expression was correlated with prognosis. Also, they found correlation between MMP-9 and Proliferating cell nuclear antigen (PCNA) expression (which is a proliferation marker) and that MMP-9 increases with cancer cells having high proliferation ability and that MMP-9 is closely associated with invasion, metastasis, and proliferation of tumor cells [31].

Conclusions

The present study can conclude that apricot oil extract has potent anticarcinogenic effect, and this novel local application of the oil may help overcoming the cyanide toxicity of its systemic ingestion and allows increasing the dose of the oil safely. Moreover, it is recommended for further studying the effect of different doses of the apricot oil extract locally and assessment of cyanide toxicity.

Acknowledgements

This work was supported by the National Research Centre. Authors' contributions: Marwa M. Ellithy, Heba E. Tarek and Heba N. Shalash conceived the study. H.E. conducted different steps of the experimental work, helped by M.E. H.S. analyzed the immunohistochemistry results. All authors shared the statistical analysis of results. Both the manuscript draft and the final one were written, revised, and approved by all authors.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021; 71:209–249.

2 Rumgay H, Murphy N, Ferrari P, Soerjomataram I. Alcohol and cancer: epidemiology and biological mechanisms. Nutrients 2021; 13:3173.

3 Gyawali B, Shimokata T, Honda K, Ando Y. Chemotherapy in locally advanced head and neck squamous cell carcinoma. Cancer Treat Rev 2016; 44:10–16.

4 Porceddu SV, Bressel M, Poulsen MG, Stoneley A, Veness MJ, Kenny LM, *et al.* Postoperative concurrent chemoradiotherapy versus postoperative radiotherapy in high-risk cutaneous squamous cell carcinoma of the head and neck: the randomized phase III TROG 05.01 Trial. J Clin Oncol 2018; 36:1275–1283.

5 Zanoni DK, Montero PH, Migliacci JC, Shah JP, Wong RJ, Ganly I, Patel SG. Survival outcomes after treatment of cancer of the oral cavity (1985–2015). Oral Oncol 2019; 90:115–121.

6 Mirabelli P, Coppola L, Salvatore M. Cancer cell lines are useful model systems for medical research. Cancers (Basel) 2019; 11: 1098.

7 Li Z, Zheng W, Wang H, Cheng Y, Fang Y, Wu F, *et al.* Application of animal models in cancer research: recent progress and future prospects. Cancer Manag Res 2021; 13:2455–2475.

8 Song Z, Xu X. Advanced research on anti-tumor effects of amygdalin. J Cancer Res Ther 2014; 10:3–7.

9 Qian L, Xie B, Wang Y, Qian J. Amygdalin-mediated inhibition of non-small cell lung cancer cell invasion in vitro. Int J Clin Exp Pathol 2015; 8:5363–5370.

10 Blaheta RA, Nelson K, Haferkamp A, Juengel E. Amygdalin, quackery or cure? Phytomedicine 2016; 23:367–376.

11 Sireesha D, Reddy BS, Reginald BA, Samatha M, Kamal F. Effect of amygdalin on oral cancer cell line: an *in vitro* study. J Oral Maxillofac Pathol 2019; 23:104–107.

12 Coletta RD, Yeudall WA, Salo T. Grand challenges in oral cancers. Front Oral Health 2020; 1:3.

13 Miranda-Filho A, Bray F. Global patterns and trends in cancers of the lip, tongue and mouth. Oral Oncol 2020; 102:104551.

14 Li Y, Zheng Y, Wang H. Anticancer activity of Vicenin-2 against 7,12 dimethylbenz[a]anthracene-induced buccal pouch carcinoma in hamsters. J Biochem Mol Toxicol 2021; 35:e22673.

15 Wali RK, Kunte DP, De La Cruz M, Tiwari AK, Brasky J, Weber CR, *et al.* Topical polyethylene glycol as a novel chemopreventive agent for oral cancer via targeting of epidermal growth factor response. PLoS One 2012; 7:e38047.

16 Badr SEA, Wahdan OA, AbdelFattah MS. Characterization and cytotoxic activity of amygdalin extracted from apricot kernels growing in Egypt. Int. Res. J. Public Environ. Health 2020; 7:37–44.

17 Saleem M, Asif J, Asif M, Saleem U. Amygdalin from apricot kernels induces apoptosis and causes cell cycle arrest in cancer cells: an updated review. Anticancer Agents Med Chem 2018; 18:1650–1655.

18 Nour A, Azar B, Rabata A, Manadili A. The effect of amygdalin in the treatment of squamous cell carcinoma induced in the buccal pouch of golden Syrian hamster. IOSR J Dent Med Sci 2016; 15:75–79.

19 Sorbye SW, Kilvaer TK, Valkov A, Donnem T, Smeland E, Al-Shibli K, *et al.* Prognostic impact of Jab1, p16, p21, p62,Ki67 and Skp2 in soft tissue sarcomas. PLoS One 2012; 7:e47068.

20 Li LT, Jiang G, Chen Q, Zheng JN. Ki67 is a promising molecular target in the diagnosis of cancer (review). Mol Med Rep 2015; 11:1566–1572.

21 Dias EP, Oliveira NSC, Serra-Campos AO, da Silva AKF, da Silva LE, Cunha KS. A novel evaluation method for Ki-67 immunostaining in paraffin-embedded tissues. Virchows Arch 2021; 479:121–131.

22 Zhang L, Hu S, Korteweg C, Chen Z, Qiu Y, Su M, Gu J. Expression of immunoglobulin G in esophageal squamous cell carcinomas and its association with tumor grade and Ki67. Hum Pathol 2012; 43:423–434.

23 Wang Z, Zhang B, Jiang L, Zeng X, Chen Y, Feng X, *et al.* RACK1, an excellent predictor for poor clinical outcome in oral squamous carcinoma, similar to Ki67. Eur J Cancer 2009; 45:490–496.

24 Sridevi U, Jain A, Nagalaxmi V, Kumar UV, Goyal S. Expression of E-cadherin in normal oral mucosa, in oral precancerous lesions and in oral carcinomas. Eur J Dent 2015; 9:364–372.

25 Moorchung N, Vasudevan B, Dinesh Kumar S, Muralidhar A. Expression of apoptosis regulating proteins p53 and bcl-2 in psoriasis. Indian J Pathol Microbiol 2015; 58:423–426.

26 Lee HM, Moon A. Amygdalin regulates apoptosis and adhesion in Hs578T triple-negative breast cancer cells. Biomol Ther (Seoul) 2016; 24:62–66.

27 Makarević J, Rutz J, Juengel E, Kaulfuss S, Tsaur I, Nelson K, *et al.* Amygdalin influences bladder cancer cell adhesion and invasion *in vitro*. PLoS One 2014; 9:e110244.

28 Ravi DK, Kumar M, Singh G, Rai SB, Chincholkar T, Saxena AK, Pandey M. Matrix Metallopeptidase 9 (MMP-9) expression in oral cancer andand adjacent normal tissue. World Journal of Surgical Medical and Radiation Oncology 2014; 3:18–27.

29 Lu X, Duan L, Xie H, Lu X, Lu D, Lu D, *et al.* Evaluation of MMP-9 and MMP-2 and their suppressor TIMP-1 and TIMP-2 in adenocarcinoma of esophagogastric junction. Onco Targets Ther 2016; 9:4343–4349.

30 Joseph C, Alsaleem M, Orah N, Narasimha PL, Miligy IM, Kurozumi S, *et al.* Elevated MMP9 expression in breast cancer is a predictor of shorter patient survival. Breast Cancer Res Treat 2020; 182:267–282.

31 Fan HX, Li HX, Chen D, Gao ZX, Zheng JH. Changes in the expression of MMP2, MMP9, and CoIIV in stromal cells in oral squamous tongue cell carcinoma: relationships and prognostic implications. J Exp Clin Cancer Res 2012; 31:90.