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Combinatorial effect of Ficus carica latex and olive oil on induced oral squamous cell carcinoma

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ABSTRACT

Abstract: The present investigation was designed to assess the protective capabilities of Ficus carica (fig) extract and olive oil, administered individually and in combination, as potent natural antiproliferative agents against the adverse effects of DMBA on the HBP.

Material and methods: Fifty five-week-old male Syrian hamsters weighing between 80 and 120 grams were split up into five groups of ten hamsters within every group: GI: left untreated, GII: 7, 12-dimethyl benz[a]anthracene (DMBA) applied topically only on three occasions per week during 14 weeks at a concentration of 0.5% in liquid paraffin, GIII: DMBA (0.5% in liquid paraffin) applied topically on three occasions per week during 14-week plus oral fig latex (50 g lyophilized fig latex in 50 ml distilled water at a dosage of 1000 mg/ml shortly prior to experimental usage). GIV: DMBA (0.5% in liquid paraffin) used topically on three occasions per week over 14-week plus oral EVOO alone (7.6 ml /kg b.w by oral

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gavage, before and/or after DMBA application. GV: DMBA applied topically (0.5% in liquid paraffin, three occasions per week during 14 weeks) alongside oral fig latex and EVOO administration using dosages and administration techniques comparable to those used in individual therapies. The Qur'anic proportion of one fig to seven olives served as the basis for the specific dosages for each fig extract and olive oil.

Results: The current research findings demonstrated that fig latex and EVOO had a positive regression impact on the growth of tumors. The combination of fig latex and EVOO significantly decreased the occurrence of OSCC (oral squamous cell carcinoma) from 100% to 0%. According to the immunohistochemical study, fig latex and EVOO significantly reduced the expression of the antiproliferative marker PCNA in comparison to GII. Such findings highlighted the chemopreventive potential of fig latex and EVOO, suggesting their roles in inhibiting tumor growth and inducing apoptosis in oral cancer cells.

Conclusion: This study underscores the value of natural dietary components in cancer prevention and supports the further exploration of fig latex and EVOO as novel, safe, and effective therapeutic agents for oral malignancies..

Keywords: **:** *HBP carcinoma, fig latex, EVOO*

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1. INTRODUCTION

Oral squamous cell carcinoma (OSCC) represents significant global public health concern because of its high prevalence worldwide. According to GLOBOCAN 2020 estimates, approximately 377,713 cases of OSCC are newly diagnosed each year, and 177,757 deaths occur annually worldwide [(1)]. In Egypt, Abd El-Aziz et al. (2020)[(2)]study among 1664 smoker participants revealed that 1.5% had OSCC and 0.12% had verrucous carcinoma.

Oral carcinogenesis is a multifactorial disease arising from the interaction of lifestyle, environmental, genetic, and epigenetic factors[(3)]. 7,12-Dimethylbenz(a)anthracene (DMBA)-induced oral carcinogenesis in golden Syrian hamsters which serves as an established widely laboratory paradigm for examining molecular, biochemical, histological, and immunohistochemical (IHC) changes [(4)]. DMBA is commonly utilized to induce tumor development in the golden Syrian hamster buccal pouch (HBP)[(5)]. HBP, a pocket-like anatomical structure, develops a well-established OSCC following repeated topical DMBA applications. DMBA initiates neoplasia by causing significant inflammation and dysplasia within the buccal pouches, alongside extensive oxidative damage to DNA [(6)]. Cumulative data indicate that DMBA-induced oral tumors share histological, morphological, biochemical, and molecular characteristics with human oral tumors [(7)].

There is considerable interest in identifying potential protective agents capable of mitigating the adverse effects linked to toxins and chemotherapy [(8, 9)]. Consequently, medicinal plants are utilized for treating various ailments and counteracting the side effects of chemotherapeutic agents, including cardiovascular and renal complications [(10, 11)].

According to the Holy Qura'n (Surah 95. At-Teen) "By the fig and the olive, By mount Sinai, By this city of security[(12)]. The olive is referenced seven times in the holy Quran—six times expressly and one time implicitly—while the fig is mentioned just once [(13)].

Within the herbal remedies that are regularly researched and shown to have advantageous anti-inflammatory, immunomodulatory, antibacterial, anticancer, chemopreventive, analgesic, and antioxidant benefits are olive (Olea Europe) and fig (Ficus carica). Oleic acid and phenolic substances are primarily responsible for their biological characteristics [(14-16)].

Fig (Ficus carica) and olive (Olea europaea) are regarded as inexpensive raw materials that serve as valuable sources for high added-value products, particularly phenolics [(17)] . Fig and olive leaves are readily accessible, low-cost natural resources that potentially contain a comparable abundance of health-promoting bioactive phytochemicals [(18)].

A mixture of figs and olives exhibited enhanced antioxidant stability and bioavailability compared to individual plant extracts. This is likely due to the greater variety of polyphenols in the mixed extracts, which can interrupt detrimental oxidative reactions by donating a hydrogen atom [(19, 20)].

There is no published report on the in vivo chemopreventive influence of consuming olive oil and fig extract separately or in combination as strong natural antiproliferative resources towards the harmful side effects of DMBA on the HBP

2. MATERIAL AND METHODS:

Chemicals: DMBA (0.5%) was purchased dissolved in paraffin oil from Sigma Aldrich. Extra Virgin Olive Oil (EVOO), certified organic (NOP/EU), with a density of 0.910 g/ml (Product No: W530191), was also purchased from Sigma Aldrich

Company (Saint Louis, MO 63103, USA).

Preparation of fig latex: Ficus carica latex was harvested by collecting drops following the incision of young fig tree leaves at a private farm in New Damietta City, Damietta, Egypt. The fresh latex was subsequently filtered using a 0.22µm filter and preserved at -20°C. For the experiment, the latex was prepared as a suspension of 50 g fig latex in 50 ml distilled water, yielding a concentration of 1000 mg/ml, immediately prior to its administration.

Animals: Fifty male Syrian hamsters five-week-old weighing between 80 and 120 grams, were taken from the animal department at Cairo University (Cairo, Egypt). The hamsters were kept in typical Sawdust-bedded enclosures having regulated humidity (30–40%), warmth (20±2°C), and a 12-hour light/dark cycle. All hamsters were provided with conventional feed and water ad libitum. A healthy animal exhibited a regular, smooth walk, clear, bright eyes, skin that is healthy, and a silky, glossy coat without swellings, injuries, parasites, and dry areas. There are no cuts or scratches on their mouths.

Sample size: According to the Abd Al-Wahab et al. (2020) investigation [21], a sample size of 10 animals per group was determined for the present investigation . This size provides 80% power to identify a 0.53 average variance at a confidence interval of 95% and a two-tailed significance level (alpha) of 0.05. In 80% of the experiments (the power), the resulting p-value was below 0.05 (two-tailed), classifying the results as "statistically significant." Conversely, in the remaining 20% of the experiments, the mean difference was considered "not statistically significant." The report was generated using GraphPad StatMate 2.00.

Experimental design: Following a one-week acclimatization period, the hamsters were randomly assigned to five groups (G(s)) of ten hamsters within every group: **GI (negative control)** left without therapy; GII (DMBA alone) 0.5% DMBA in paraffin oil applied topically to the right HBP utilizing a size 4 camel hair brush, on three occasions weekly during 14 weeks [21]. **GIII (fig latex)** DMBA applied topically (as GII) plus oral gavage of fig latex suspension (1000 mg/ml concentration of 50 g lyophilized fig latex in 50 ml distilled water) immediately before experimental use, administered before and/or after DMBA application [22]. **GIV (EVOO):** Topical application of DMBA (as GII) + Oral administration of EVOO alone (7.6 ml /kg b.w by oral gavage, before and/or after DMBA application)[22]. **GV (fig latex - EVOO)** received topical DMBA (as GII) plus oral gavage of fig latex and EVOO, using the same doses and administration protocol as the individual treatments. The chosen doses of fig extract and olive oil were based on the Qur'anic ratio of one fig to seven olives.

General health assessments: Throughout the trial, changeover in the animals' overall health status were documented. Indicators of illness or injury in animals included one or more of the following symptoms: anorexia, lethargy, corner huddling, sneezing, wheezing, nasal or ocular discharge, perianal wetness, diarrhea, and alopecia. Animal body weights were recorded weekly during the experimental period.

Investigations: Upon completion of the trial, gross findings (including size of the mucosa, exudation, ulceration, and tumors) were documented. The hamsters were then euthanized, the right cheek pouch was removed, and the size of every tumor was calculated using a Vernier caliper. Tumor volume (Vmm^3) was determined using the formula $Vmm^3 = (4/3)\ \pi$ [(D1/2) (D2/2) (D3/2)], where D1, D2, and D3 represent the three diameters (in mm) of the tumor [23]. Subsequently, the right cheek pouch was removed, fixed in 10% neutral buffered formalin, and treated and inserted into paraffin blocks on a regular basis for subsequent histological and immunohistochemical analysis.

Histopathological examinations: The preserved samples were incorporated in paraffin wax to create paraffin blocks after being dehydrated in an increasing ethanol sequence. A rotary microtome was utilized to generate 4µm-thick tissue slices, which were then placed on glass slides, processed, and stained using hematoxylin and eosin (H&E) to light microscopic analysis.

Calculation of the Depth of Invasion (DOI): Depending on the H&E slide, DOI was calculated for every surgical sample. DOI was calculated from the lowest point of tumor penetration to the basal layer of the surface epithelium. The American Joint Committee on Cancer (AJCC) has additionally classed it as less invasive (≤5 mm), moderately invasive (6–10 mm), and highly invasive (≥10 mm) [24] (Fig.1). The Leica QWIN V3 computer program for photo analysis (Switzerland), was used to calculate the DOI. This was carried out in the Department of Oral and Dental Pathology, Faculty of Dental Medicine (Boys-Cairo), Al-Azhar University, Egypt.

Immunohistochemical examination: To show the expression of PCNA antibodies, additional tissue slices were sliced and subjected to the typical marked streptavidin-biotin procedure. The tissue slices fixed in paraffin were dewaxed and rehydrated using distilled water and graded ethanol. After ten minutes of treatment with 3% H2O2 in methanol, endogenous peroxidase was inhibited. After applying citrate buffer solution (pH 6.0) and microwaving for three periods of five minutes at 95°C, the antigen was recovered. Phosphate-buffered saline (PBS) was then used for rinsing. Next, single or double drops of the main antibody (PCNA) diluted 1:100 in Tris buffer solution were added to the tissue sections. They spent the entire night at normal temperature at 4°C in a humid environment. Following a PBS wash, a biotinylated secondary antibody was incorporated and allowed to sit at an ambient temperature for 30 minutes. Tissue slices were rinsed with PBS and then exposed to diaminobenzidine (Sigma, USA) for two to four minutes in order to produce color. Once the

desired color strength had been attained, the slides were cleaned, Hematoxylin was used as a counterstain, and after that a mounting media was applied.

To ascertain the occurrence of positive instances and the exact spots of immunostaining within the tissues. A light microscope was used to examine the immune-stained slices. Additionally, the area percentage of immunostaining-positive cells was calculated using a picture analytics computer program.

Statistical analysis: The findings were statistically assessed and documented as the mean ± standard deviation (SD). SPSS version 17.0 for Windows was utilized to perform a one-way test of variation (ANOVA). ANOVA was utilized to compare greater than two independent groups utilizing quantitative data and parametric distribution. The LSD test was then used for post hoc analysis. $P > 0.05$ was deemed non-significant, $p < 0.05$ was deemed significant, and $p < 0.001$ was deemed extremely significant.

3. RESULTS:

Gross observations and tumor volume: GI (negative group) Animals showed no gross alterations, maintaining healthy and active behavior; the length of both buccal pouches was approximately 5 cm. The right HBP mucosa was pink, surface was smoothness, and don't have notable abnormalities **(Fig.2A)**. **GII** (DMBA-treated group) Animals exuded a foul odor and white particles from their mouths. All hamsters had noticeable perioral hair loss, which extended up to the belly in some animals with substantial body weight. This might have been brought on by inadequate food intake as a result of DMBA-caused oral cavity inflammation. The pouch depth started to drop by up to 2 cm and didn't change till the trial was over. The pouch depth began to decrease up to 2 cm and remained fixed until the end of the experiment. The animals were debilitated and skinny. The right HBP mucosa showed a whitish membrane and granular surface on the pouch mucosa, erythema of various degrees, and numerous elevated nodules also present. Using vernier caliper, hamsters with tumors had an average tumor volume of 775.5 mm^3 **(Fig.2B)**. In **GIII** (fig latex treated group) and **GIV** (EVOO treated group), HBP mucosa showed variable changes, including different exophytic tumor mass, erythematous mucosal surface to usual regular color, and the hamsters seemed healthy. Using vernier caliper, the average tumor volume in hamsters with tumors in GIII and GIV were 93.8 mm^3 and 90.2 mm^3 respectively **(Fig.2C&D)**. **GV (combination group)** The hamsters seemed healthy, and the erythematous mucosal surface of the HBP mucosa changed to a standard typical hue having a smooth surface. (**Fig.2E)**. General health examinations and common clinical findings observed within the present research groups are summarized in Table 1.

Table 1: Typical clinical results seen in the groups under study.

features	Groups				
	GI	GII	GIII	GIV	GV
loss of appetite	-	+++	+	+	-
Inactivity	-	+++	+	+	-
corner huddling	-	+++	+	+	-
sneezing, wheezing, and/or discharge from the nose or eyes,	-	+++	+	+	-
wetness around the tail, diarrhea	-	+++	+	+	-
hair loss	-	+++	+	+	-
Gross observations					
Papillomatous lesion	-	+++	+	+	-
Pouch length	5 cm	1.5-2cm	4cm	4cm	4.5cm

Ulcers	-	+++	+	+	-
Exudation	-	+++	+	+	-
Tumors	-	100 %	30 %	30 %	-

– = no change; + = mild; ++ = moderate; +++ = severe

Statistical analysis results, with regard to the tumor volume, revealed that GII recorded greatly significant variation with GIII, GIV and GV ($p < 0.001$). Furthermore, GIII, GIV and GV displayed non-significant difference when comparing to each other (p value > 0.05). **(Table 2, Fig.1).**

Statistical analysis results, with regard to body weight, revealed that, there was greatly significant increase in GI, GIII, GIV and GV when compared with GII ($p < 0.001$). Moreover, no significant variations were shown amongst other groups (p value > 0.05). **(Table 2, Fig.1).**

Table.2: Comparison across examined groups as regard tumor volume and Body weight.

		GI (n = 10)	GII (n = 10)	GIII (n = 10)	GIV (n = 10	GV (n = 10	F	P-value
Tumor volume	**Mean**	-----	775.5	93.8	**90.2**	-	**134.8**	**< 0.001 HS**
	±SD	-----	109.3	47.1	**40.5**	-		
Body weight	**Mean**	495	296	412	**420**	**487**	**10.11**	**< 0.001 HS**
	±SD	18	13.3	16.1	**22**	**17**		

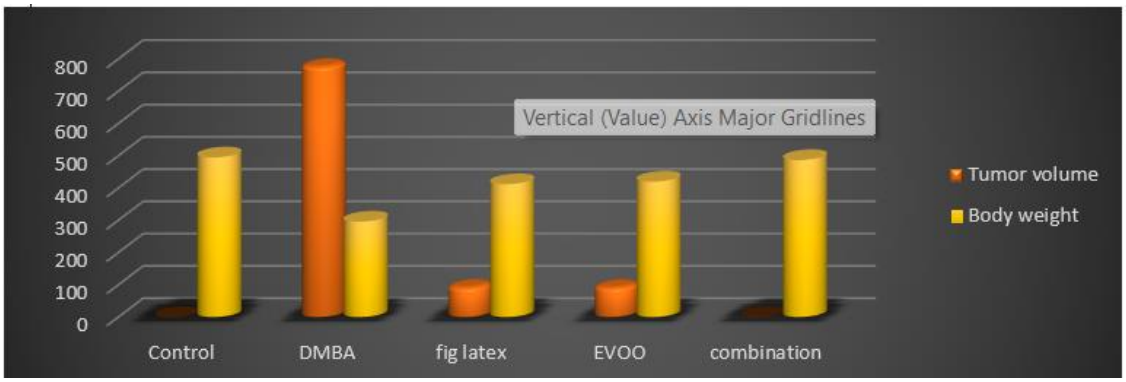


Fig.1: Bar chart representing tumor volume and body weight in the studied groups

Histopathologic findings and immunohistochemical (IHC) results: GI The HBP mucosa's lining epithelium was extremely thin, having keratinized stratified squamous epithelium that is three to five cells thick, according to H&E staining. One to two layers of polyhedral spinous cells, one layer of cuboidal basal cells, and flattened granular cells with small keratohyalin granules were visible in the epithelial layers. The epithelium was sparsely coated with keratin. The epithelium connective tissue interaction was reasonably flat without rete processes. The submucosa was composed of delicate and loose connective tissue and a layer of striated muscle fibers **(Fig. 2F)**. The IHC staining in **GI** showed that although a small number of PCNA-positive cells were discovered and dispersed across the basal cell layer, PCNA antigen was only isolated in the nuclei of epithelial cells. The cytoplasm showed no response. PCNA exhibited positive nuclear expression (10.14%) throughout the basal cell layer **(Fig.3A).**

In **GII,** the H&E stain indicated that two instances had mild SCC, and eight cases had well-differentiated SCC. Histologically, well-differentiated SCC shows malignant epithelial cells that penetrate extensively through the connective tissue (DOI=11.3mm). The invading epithelial cells were observed as cell nests or as detached and scattered cells, with confirmation of keratinization. The nuclear/cytoplasmic ratio was demonstrated in tumor cells having pleomorphic,

hyperchromatic nuclei. **(Fig.2G)**. In **GII,** the IHC staining utilizing PCNA showed positive nuclear expression (72.53%) across the layers of the epithelium **(Fig.3B)**.

In **GIII** and **GIV**, the H&E stain revealed exhibited hyperkeratosis to mild epithelial dysplasia in two hamsters. While six animals had moderate to severe epithelial dysplasia alongside top-to-bottom alterations or carcinoma in situ (CIS), whereas two animals had superficial invasion of malignant cells in a type of well-differentiated SCC that was juxtapithelial and was not spreading to the deeper C.T. (DOI=0.9-1.1mm). **(Fig.2H&I)**. In **GIII** and **GIV**, the IHC staining via PCNA showed positive nuclear expression (33.5% and 30.22% respectively) across the layers of the epithelium **(Fig.3C&D)**.

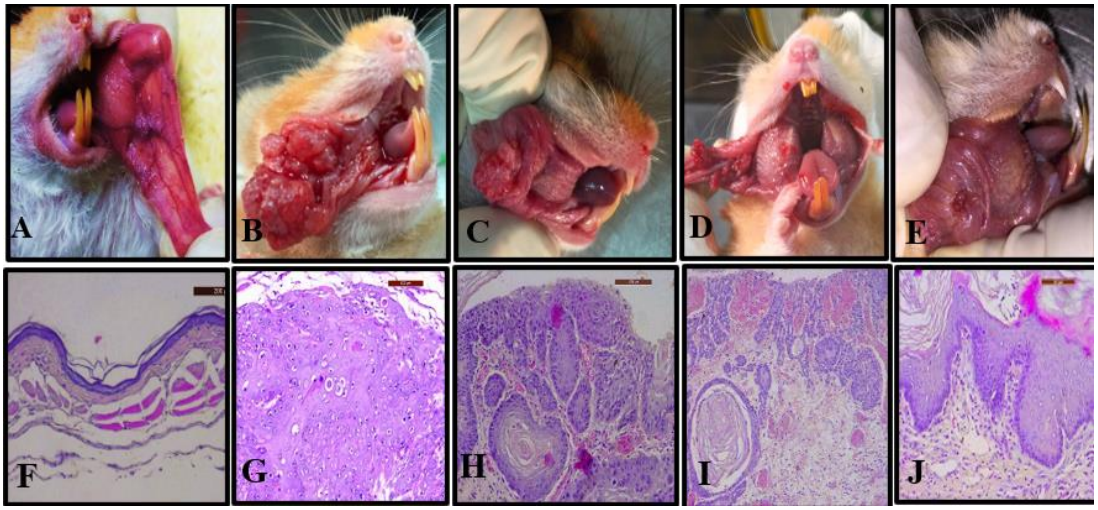
In **GV** four animals displayed mild to moderate epithelial dysplasia, characterized by focal dysplastic areas within the epithelium, including hyperchromatism, altered nuclear/cytoplasmic (N/C) ratio, cellular and nuclear pleomorphism, prominent nucleoli, and multiple foci of group cell keratinization. The remaining six animals showed hyperkeratosis with normal epithelium. **(Fig.2J)**. In **GV**, the IHC staining utilizing PCNA showed positive nuclear expression (21.41%) across the layers of the epithelium **(Fig.3E)**. Histopathological alterations in the HBP of the control and treated animals among the studied groups are summarized in Table 3.

Table 3: Histopathological alterations observed in the HBP of the control and treated animals across the study groups.

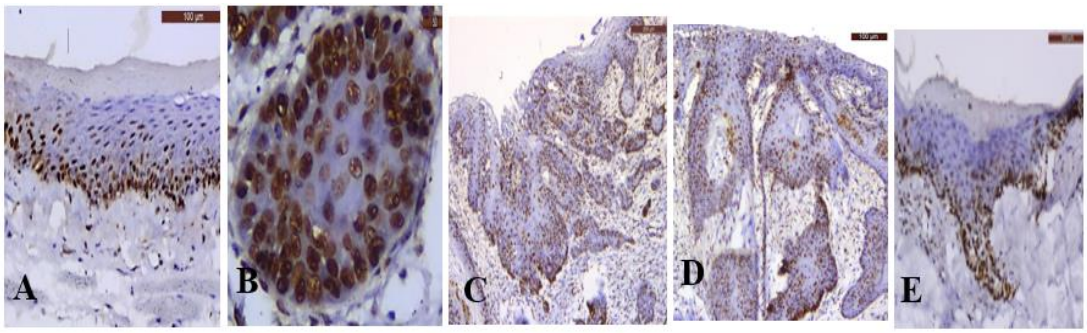
Features	Groups				
	GI	GII	GIII	GIV	GV
Irregular epithelial stratification	-	+++	+	+	+
Basal cell hyperplasia	-	+++	+	+	-
loss of basal cells polarity	-	+++	+	+	-
Individual cell keratinization	-	+++	+	+	+
keratin pearls	-	+++	+	+	-
Nuclear pleomorphism	-	+++	+	+	+
Cellular pleomorphism	-	+++	+	+	+
Abnormal mitotic figures	-	+++	+	+	+
Hyperchromatism	-	+++	+	+	+
Abnormal nucleus/cytoplasm ratio	-	+++	+	+	+
Inflammatory infiltration	-	+++	+	+	-
Increase amount of collagen fibers	-	-	+	+	
DOI	-	11.3mm	0.9mm	1.1mm	-
Epithelial histological changes					
Mild dysplasia	-	+++	20%	20%	40%
Moderate dysplasia	-	+++	40%	30%	-

Severe dysplasia	-	+++	10%	20%	-
CIS	-	+++	20%	10%	-
SCC	-	Well (70 %) Moderate (50 %)	Well (20 %)	Well (20 %)	-

− = no.change; + = mild; ++ = moderate; +++ = severe.



(Fig.2A): Photograph of GI (normal). Displaying a rosy, smooth buccal pouch mucosa. **(Fig.2B):** Photograph of GII (DMBA) several polypoid papillary tumor masses enveloped by hemorrhage spots. **(Fig.2C):** Photograph of GIII (fig latex group) mucosa showed varied shifts, including smooth, normal-colored, reddened mucosal surface. **(Fig.2D):** Photograph of GIV (EVOO group) displaying a little lump that is neither bleeding or ulcerating. **(Fig.2E):** Photograph of GV (combination group) displaying a significant reduction in tumor mass size without bleeding or ulceration. **(Fig.2F):** Photomicrograph of GI (normal) displaying : The epithelium is composed of 2-4 layers: 5 superficial keratinized squamous cells with flattened rete ridges, the C.T layer, the muscular layer, and the deep layer of loose areolar connective tissue. (H&E stain X200) **(Fig.2G):** Photomicrograph of GII (DMBA) displaying moderately-differentiated SCC with deeply invasive tumor islands into the connective tissue. (H&E stain X200). **(Fig.2H):** Photomicrograph of GIII (fig latex group) displaying well-differentiated SCC having superficial invasion of tumor islands into the connective tissue. (H&E stain X200). **(Fig.2I):** Photomicrograph of GIV (EVOO group) displaying well-differentiated SCC with superficial invasion of tumor islands into the connective tissue. (H&E stain X200). **(Fig.2J):** Photomicrograph of GV (combination group) showing mild to moderate epithelial dysplasia. (H&E stain X200).



(Fig.3A): IHC expression of PCNA in GI shows positive nuclear expression in basal epithelial layers. **(Fig.3B):** IHC expression of PCNA in GII displaying positive nuclear expression across the tumor cells. (**Fig.3C):** IHC expression of

PCNA in GIII displaying positive nuclear expression across the layers of the epithelium. (**Fig.3D):** IHC expression of PCNA in GIV displaying positive nuclear expression across the layers of the epithelium. (**Fig.3E):** IHC expression of PCNA in GV displaying positive nuclear expression across the layers of the epithelium.

Statistical analysis findings of PCNA expression and DOI:

According to the findings of statistical analysis, GI had the smallest mean area percentages (10.14%) and GII had the greatest mean area percentages (72.53%) for PCNA expression. **(Table 4, Figure 4).**

The DMBA treated group (GII) when compared with either GI (left untreated) or chemopreventive groups (GIII, GIV and GV) , there was highly significant variation; (p value< 0.001). Furthermore, GIII, GIV and GV showed non-significant difference when comparing to each other (p value > 0.05). **(Table 5).**

The findings of the statistical analysis showed that GII had the greatest mean area percentages (11.3%) and GIII had the smallest mean area percentages (0.9%) in terms of DOI expression **(Table 4, Figure 4).**

There was a highly significant variation (p value<0.001) between the DMBA-treated group (GII) and either the GI (left untreated) or chemopreventive groups (GIII, GIV, and GV). Furthermore, GIII, GIV and GV showed non-significant difference when comparing to each other (p value > 0.05). **(Table 5).**

Table 4: DOI and PCNA expression comparisons across the groups under study.

		GI (n = 10)	GII (n = 10)	GIII (n = 10)	GIV (n = 10)	GV (n = 10)	F	P-value
PCNA	**Mean**	10.14	72.53	33.5	30.22	21.41	**11.02**	**< 0.001 HS**
	±SD	2.16	12.54	16.1	12.2	8.7		
DOI	**Mean**	-	11.3	0.9	1.1	0	**180.7**	**< 0.001 HS**
	±SD	-	2.5	0.88	1.07	0		

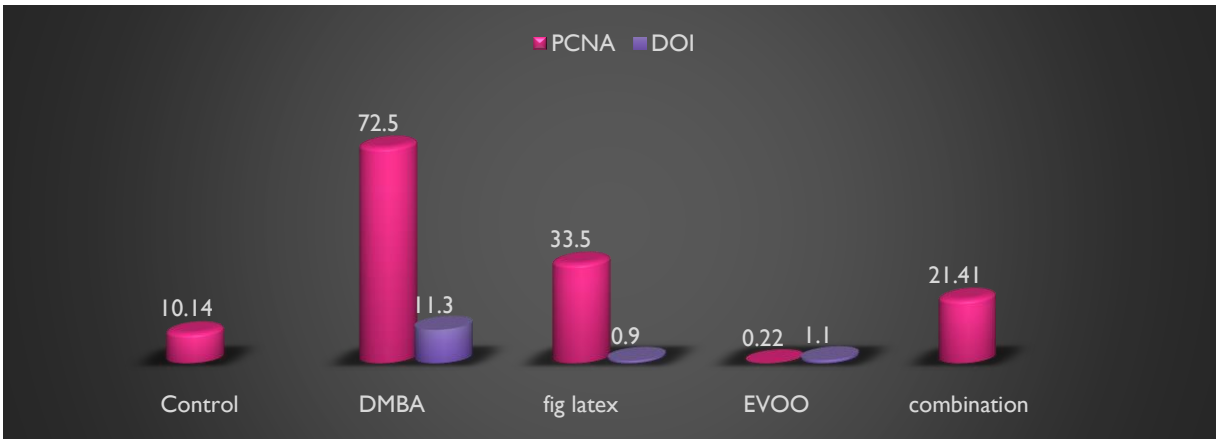


Fig.4: Bar chart representing PCNA experssion and DOI in the studied groups.

Table 5: Comparing the groups under study in terms of PCNA

	PCNA		P-value for post analysis using LSD test				
	Mean ± SD	Range	G1	GII	GIII	GIV	GV
Group I	10.14 ± 2.16	7.2 – 13.2	--	0.000	0.000	0.000	0.000
Group II	72.53 ± 12.54	71.3 – 79.3	0.000	--	0.000	0.000	0.000
Group III	33.5 ± 16.1	28.9 –37.3	0.000	0.000	--	0.125	0.117
Group IV	30..22 ± 12.2	25.4 – 34.2	0.000	0.000	0.125	--	0.111
Group V	21.41 ± 8.7	16.9 –27.3	0.000	0.000	0.117	0.111	--
F	**11.02**						
P-value	**<0.001 (HS)**						

4. DISCUSSION:

Animal models are thought to be crucial for studying the course of illnesses and the development of diagnostic or treatment procedures. There has been increased interest in the utilization of herbal remedies for the chemoprevention and treatment of oral cancer, including green tea and fruit and vegetable preparations from grape, apple, and tomato [25-27]. To present, the anticancer efficacy of fig latex and EVOO, a therapeutic natural plant, on oral cancer hadn't been documented. The chemopreventive properties of fig latex and EVOO were investigated in this work utilizing an animal model that was developed for mouth cancer utilizing DMBA.

Combining therapy of two or three polyphenols reduced the development of cancer more successfully than a single chemical therapy, according to preclinical research on the anticancer impact of polyphenols from various plants. numerous metabolic pathways implicated in carcinogenesis might be pleiotropically affected by the synergistic impact of numerous nutrients, which can also influence the bioavailability of natural substances [28, 29]. Thus, using an animal model of oral cancer, we assessed the impact of fig latex and EVOO on cancer cells. The current research demonstrated the inhibitory impact of fig latex and EVOO on the growth of malignant tumors by comparing average tumor size and IHC staining using PCNA between the fig latex-treated, EVOO- treated either alone or combined and cancerous group. Based on available English literature, this represents the first investigation to evaluate fig latex and EVOO as a novel chemopreventive strategy for DMBA-induced HBP carcinogenesis.

According to reports, the OSCC occurrence in the HBP was highly replicable (100%) after 14 weeks of DMBA (0.5%) treatment in hamsters [26]. DMBA can cause various dysplastic and neoplastic lesions having molecular and morphological changes which can be connected to human carcinogenesis, according to the dosage and time of the carcinogen being delivered [30]. After 14 weeks of cancer initiation, DMBA caused precancerous and cancerous morphological alterations on the HBP within the present investigation.

DMBA treatment to induce cancer in animals may additionally contribute to various clinical findings including loss of appetite, Inactivity, coughing, sneezing, and/or nasal or ocular discharge, hair loss as well as substantial body weight loss, not being able to eat, an absence of hunger, and an elevated rate of metabolism due to the occurrence of oral cancer [25, 31]. The use of DMBA in this investigation resulted in a notable decrease in body weight and great changes in the animal's overall health of the cancer-induced animals, which is consistent with other results. However, the fig latex and/or EVOO therapy regulated the weight loss.

Ghandehari et al (2018)[32]stated that, cancer cells have been shown to be able to avoid apoptosis and to fail at any stage of the cell cycle. Therefore, it is probable that proliferation of cells and tumor diameters are decreasing because to the activation of apoptosis with fig latex and EVOO with cell cycle. In the current study, fig latex and EVOO decreased the tumor voulmes of fig latex and EVOO treated animals either alone or combined with highly significant reduction ($p < 0.001$) in comparison to the tumor sizes in the malignant group of hamsters.

Our results revealed that fig latex and EVOO were able to downregulate PCNA expression either alone or combined with significant variation in comparison to GII (p value < 0.001). In accordance, fig latex and EVOO have been researched as a possible antioxidant and anti-inflammatory medications due to their polyphenol compositions which trigger apoptosis and prevent cell division [33, 34]. Numerous plants showed antitoxic action for various types of cancer, such as the colon, ovary, liver, kidney, central nervous system, and stomach cells exposed to various toxic chemicals, according to reporters

experiments [35, 36]. These researchers attributed this effect to the antiproliferative properties of the plants. Additionally, the synergies between fig latex and EVOO may contribute to their antitumor effectiveness [11, 22, 33, 34]. Furthermore, hydroxytyrosol, one of the strongest antioxidants in olive oil, and oleuropein, the main phenol in olive goods, could further work by altering oncogenic signaling pathways, which results in cell death [37]. Furthermore, only a small number of investigations show that the various constituents of fig latex might inhibit and reduce inflammation and tumor development [32, 38, 39]. AlGhalban et al (2021)[39] stated that, fig latex exhibits diverse molecular mechanisms of action, such as antiproliferative and antimetastatic effects, as well as notable impacts on cellular morphology. Additionally, Ghandehari et al (2018)[32] found that, fig latex protects against the damaging effects of oxidative stress brought on by free radicals generated by immune system cells.

According to Tezcan et al (2015)[40] and Tezcan et al (2019)[41], the expression of lethal-7d (let-7d) is induced by Fig latex and oleuropein (olive oil extract), which in turn prevents invasion. Additionally, it was found that the use of fig latex and olive oil extract have an inhibitory effect on the growth of endothelial cells cultivated in a collagen matrix-containing three-dimensional medium.

Fig latex and EVOO have been found to possess the ability to impede the growth of cancerous cell lines through various mechanisms[42-44]. Notably, this effect was observed without any accompanying cytotoxicity towards normal human cells. Fig latex and EVOO have been found to possess antiproliferative effects on cancer cells, specifically its ability to induce apoptosis through modulation of the cell cycle. This is achieved by increasing the population of cells in the G0/G1 phase while decreasing the population in the S and G2/M phases[32, 45]. According to Han et al (2023)[45] and Shin et al (2017)[46], another possible mechanism for these effects could be connected to the inhibition of DNA synthesis, the beginning of apoptosis, the arrest of the cell cycle in cancerous cells, and activation of both the extrinsic death receptor and the intrinsic mitochondrial-dependent apoptosis signaling pathway.

Unexpectedly, blending fig with EVOO offers a novel cure that is enriched in polyphenolic compounds, capable of contributing to a hydrogen atom and preventing the adverse reaction activities. In addition to attenuating cardiac and renal toxicity, this argued therapy additionally demonstrated strong anti-inflammatory, antioxidant, and anti-apoptotic properties. As a result, it gained considerable interest for usage in avoiding some common chronic illnesses, such as diabetes and hepatic diseases [11, 19, 47].

5. CONCLUSIONS

Based on the current findings, we conclude that fig (Ficus carica) tree latex and EVOO are promising candidates as potential agents for inhibiting the proliferation and development of cancerous cells, and may demonstrate comparable effectiveness in clinical investigations

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