

## CHEMOPREVENTIVE EFFECT OF OLIVE OIL IN EXPERIMENTALLY INDUCED HAMSTER BUCCAL POUCH EPITHELIAL DYSPLASIA (RANDOMIZED CONTROL TRIAL)

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### ABSTRACT

The progression of tumor depends on the level of Bcl-2 which protects the cells from undergoing apoptosis, and its level should be more than the level of the death inducer. So, our **Aim:** To investigate chemopreventive effect of olive oil in epithelial alteration which induced in hamster buccal pouch.

**Methods:** Epithelial dysplasia will occur by the application of DMBA on alternative days for 8 weeks on hamster buccal pouch. Hematoxylin and Eosin (H&E) and Streptavidin-biotin immunoperoxidase staining techniques were used to detect the expression of Bcl-2 in three groups (G1 negative control or normal group, G2 DMBA group and G3 olive oil chemoprevention group). Evaluation of the immunostaining was done by using computer image analyzer system.

**Results:** H & E stained histological section in G1 with normal mucosa of HBP intact, and continuous epithelium of thin keratinized stratified squamous epithelium while in G2 there were incidence of severe epithelial dysplasia with hyperkeratosis & G3 reduced dysplasia incidence and severity as compared to G2. Bcl-2 immunohistochemical expression in G1 in basal & suprabasal layers while in G2 & G3 located in all epithelial layers with statistically highly significant difference ( $P < 0.01$ ) in means values of Bcl-2 expression among ( $G2 > G1$ ,  $G3$ ) and ( $G3 > G1$ ).

**Conclusion:** Olive oil plays an important role in reduction and prevention of transformation of normal oral epithelium into epithelial dysplasia. The liquid form of olive oil may be considered as a new line of chemoprevention in oral dysplasia and the Bcl-2 expression evidenced this reduction of abnormality in epithelial dysplasia.

**KEYWORDS:** Oral epithelial dysplasia, chemopreventive effect, olive oil, bcl2, induced hamster dysplasia.

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## INTRODUCTION

In gauge medical nomenclature, abnormality of development means dysplasia, but the histological features of dysplasia characterized by epithelial cellular and structural changes <sup>(1)</sup>. The most common sign of premalignant lesions is dysplastic changes which is considered as the main histopathological features of epithelial premalignant lesions <sup>(2)</sup>. Abnormal activity in normal epithelium is considered as a histopathological feature of dysplasia. The term dysplasia was inserted by Reagon in 1958 in relation to cells exfoliated from the uterine cervix <sup>(3)</sup>. The existence of epithelial dysplasia play an important role in predicting malignant progression than the clinical characteristics <sup>(4,5)</sup>. The progression of cancer in the epithelium of the upper aerodigestive tract is believed to be associated with the presence of dysplastic areas. The cellular atypia and wastage of normal ripeness and stratification are the common characteristic histopathological dysplastic features of a stratified squamous epithelium <sup>(6)</sup>. The transformation to cancer is believed to be associated with the presence of dysplastic areas in the oral mucosa. Interestingly, the rate of mild epithelial dysplasia transformation to malignant form is similar to that of severe dysplasia <sup>(7)</sup>. The suppression of these lesions to transform into malignant tumors is considered as the essential goal of management of pre-malignant lesions. Until now there is no solidarity about what the best treatment of oral dysplasia to inhibit the progression of malignancies. Cancer is considered as one of the most horrible diseases and act as the 2nd major cause of death all over the world with circa 14 million new cases and 8.2 million cancer-related deaths in 2011 <sup>(8)</sup>. Diet play an important role in cancer and computation for about 30-35% of cancer jeopardy so cancer could be effectively inhibited by modifying dietary patterns <sup>(9)</sup>. One of the fundamentals of human diet are Mediterranean dietary fats (MDF) and the essential ingredient of dietary fat is olive oil. Since ancient time different cultures believe that, the olive

fruit has been looked upon as a signal of hope, peace and sacredness <sup>(10)</sup>. Olive oil (Oleaeuropaea, Oleaceae), virgin olive oil and extra virgin olive oil is an essential ingredient of the MDF; it is a mix of fatty acids such as oleic and linoleic acid, secoiridoids (oleuropein and oleocanthal), simple phenols (tyrosol and hydroxytyrosol), lignans (pinorexinol), flavonoids (apigenin), hydrocarbons (squalene), triterpenes (maslinic acid), and phytosterols ( $\beta$ -sitosterol). The extraction technique applied to obtain oil from the olives play an important role in chemical structure of olive oil. By applying high pressure, the olive oil is separated from the fruit core after olives crushed. Throwing, post-pression or re-pression, with or without the use of hot water is considered as additional processes. But in these additional methods the olive oil shows a sturdy color, feeble flavour, and a higher concentration of free fatty acids <sup>(11,12)</sup>. Olive oil is including two main fractions, major fraction and minor fraction. By weight, the major fraction represents more than 98% of oil and mostly consists of mono- and poly-unsaturated fatty acids. Fatty acid component of other seed oils is completely various from that of olive oil. The concentration of oleic acid in olive oil ranges from 56%-84% of total fatty acids which considered as one of mono-unsaturated fatty acids (MUFAs) that present in high amount in olive oil <sup>(13)</sup>. Also, olive oil contains very small amounts of saturated fatty acids such as stearic, palmitic acids (8%-14%) and omega-3 fatty acids such as  $\alpha$ -linolenic acids <sup>(13,14)</sup>.

The living cells contain genes that normally play a role in the regulation of cell proliferation and function if these cells exposed to mutational alteration lead to uncontrolled cell growth thus leading to cancer. The genes implicated in tumorigenesis include the regulatory genes which regulate the cell proliferation (either promoting or inhibiting), apoptotic genes that controlled cell death and finally genes which responsible for repair of damaged DNA. Reliant on how they influence every process, these genes are named as

tumor suppressor genes (growth inhibitory), proto-oncogenes (growth promoting), or anti-apoptotic genes (inhibits apoptosis)<sup>(15)</sup>. One of anti-apoptotic protein is B-cell lymphoma/leukemia-2 (Bcl-2) that interacts with and is regulated by p53. The progression of tumor depends on the level of Bcl-2 which protects the cells from undergoing apoptosis, and its level should be more than the level of the death inducer, bax<sup>(16)</sup>. The present study was carried out to investigate chemopreventive effect of olive oil in epithelial alteration (Epithelial dysplasia) & carcinogenesis which induced in hamster buccal pouch.

## MATERIAL AND METHODS

15 golden Syrian male hamsters five weeks old, by weighting 80-120 gms were used as experimental animals. They were obtained from animal house, Faculty of Pharmacy, Cairo, Boys Al Azhar University. The experiment was carried out according to the ethical & research committee protocol of the Faculty of Pharmacy, Cairo, Boys Al Azhar University. The procedure were conducted in accordance with the committee for purpose of control and supervision on experiment on animals (CPCSEA Guide lines)<sup>(17)</sup> The animals were randomly divided into three groups (G1, G2 and G3): **G1** (negative control or normal group): 5 animals were left untreated. **G2** (DMBA: 2,4-Dimethoxybenzaldehyde group): 5 hamsters were included in this group, the right hamster buccal pouch (HBP) of these hamsters were painted with 0.5 DMBA in paraffin using a number 4 camel hair brush three times a week for 8 weeks. **G3** (olive oil chemoprevention group) 5 hamsters were included in this group were received dose of olive oil (1ml/100 g body weight)<sup>(18)</sup> given by the oral route using a specific vehicle one week before, as well as during the application of DMBA on alternative days for 8 weeks. After termination of the experiment, all animals were euthanized, and the buccal mucosa was excised and prepared for both histological

examination by H & E and immunohistochemical examination by Bcl-2. The immunostaining procedures by H & E and immunohistochemically by Bcl-2 the immunostaining procedure was performed according to the manufacturer instructions<sup>(19)</sup>. The immunostained sections were examined using light microscope to assess the prevalence of positive cases and the localization of immunostaining within the tissues. In addition, image analysis computer system was used to assess area percentage of positive cells of the immunostaining. This was done in the Oral and Dental Pathology Department - Faculty of Dental Medicine - Boys- Cairo - Al-Azhar University. The degree of positive staining for each antibody was evaluated by a well-established semi-quantitative scoring on a scale range from negative to strong positive staining as follow: Strong staining (more than 50% stained), moderate staining (between 25 and 50% stained), weak staining (between 5 and 25% stained), and negative (less than 5% stained)<sup>(20)</sup>. Statistical presentation and analysis of the present study was conducted, using the mean, standard deviation, ANOVA and Tukey's test by SPSS V204. Significant level: Non-Significant >0.05 Significant <0.05\* High Significant <0.01\*.

## RESULTS

**Group1(Normalgroup):** Normal clinical features of HBP mucosae were pink in color with smooth surface and no observable abnormalities (Fig.1.A). H & E stained histological section of G1 showed, normal mucosa of HBP intact, and continuous epithelium composed of thin keratinized stratified squamous epithelium. Subepithelial connective tissue (C.T), muscular layer and areolar layer were seen (Fig.1.B). Bcl-2 immunohistochemical stained section of G1 showed weak (6.710 %) positive expression which limited to basal and suprabasal layers (Fig.1.C). **Group 2** (DMBA group): Clinical picture of HBP showed tumor mass and ulcerative lesion in mucosa (Fig.2.A). H&E

stained histological section of G2 showed 100% of cases incidence of severe epithelial dysplasia with hyperkeratosis, acanthosis, elongation and broad rete pegs with criteria of dysplasia, included loss of adhesion, hyperchromatism and abnormal mitosis with intact basement membrane (Fig.2.B). Immunohistochemical stained (Bcl-2) section of G2 showed strongly positive staining (53.45 %) which located in all epithelial layers (Fig.2.C). **Group 3** (chemoprevention group): Clinical picture of HBP showed white discoloration without ulceration and bleeding (Fig.3.A). H&E stained histological section of G3 showed reduced epithelial dysplasia incidence and severity as compared to G2, Three exhibited mild epithelial dysplasia while two specimen appeared apparently normal almost

the same as G1 (Fig.3.B). Immunohistochemical stained (Bcl-2) section of G3 showed mild (weak) positive staining (53.45 %) which located in all epithelial layers (Fig.3.C).

### Statistical analysis

Results were revealed that, in regard to expression of Bcl-2, G2 had recorded the highest mean area percentage (53.45%), while G1 had recorded the lowest mean area percentage (6.710%) and there was high significant difference between G1, G2, and G3 where P value was  $<0.01$ , also there was high significant difference between the following groups (G1 and G2), (G2 and G3) and (G1 and G3), respectively where P value was ( $<0.01$ ) (Table 1, Chart 1).

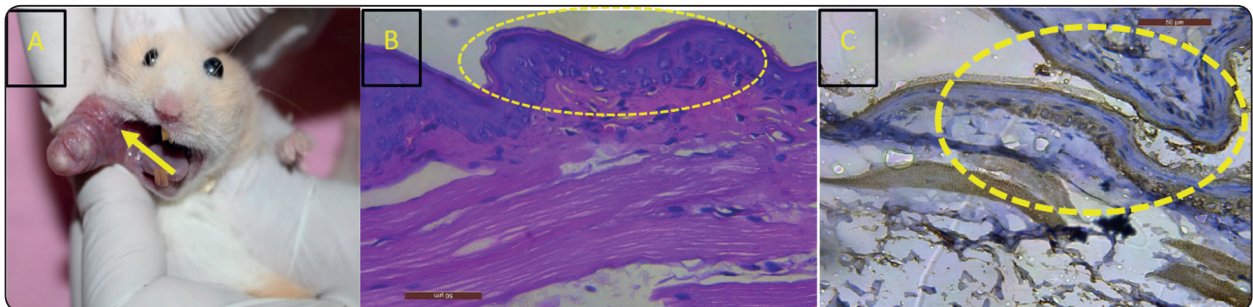


Fig. 1 A) Clinical features of G1 the yellow arrows focused on HBP mucosae were pink in color with smooth surface. B) H & E histological section of G1 the yellow dotted area focused on, intact, and continuous epithelium of HBP mucosae (X 200). C) Bcl-2 immunohistochemical stained section of G1 the yellow dotted area focused on weak staining which limited in basal and suprabasal layers (X 200).

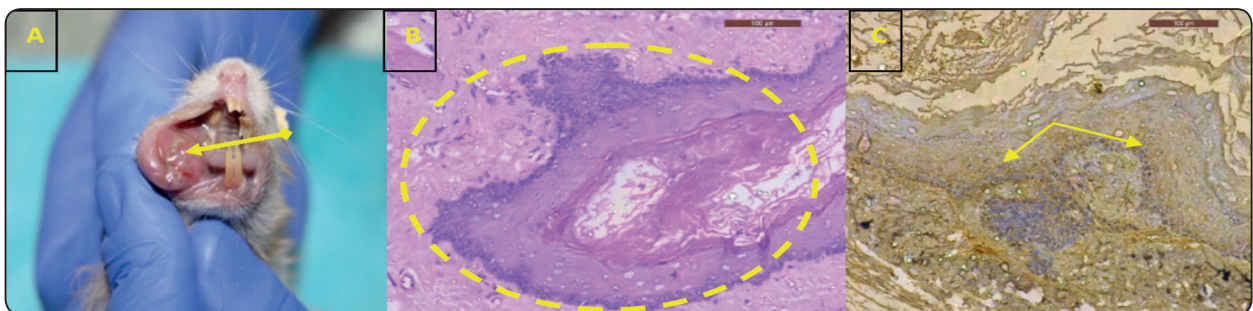


Fig. 2 A) Clinical picture of G2 the yellow arrows focused on HBP with tumor mass and ulcerative lesion in mucosa. B) H&E stained histological section of G2 the yellow dotted area focused on severe epithelial dysplasia with hyperkeratosis, acanthosis, elongation and broad rete pegs with criteria of epithelial dysplasia (X 200). C) Immunohistochemical stained (Bcl-2) section of G2 the yellow arrows focused on strongly positive staining in all epithelial layers (arrow) (X 200).



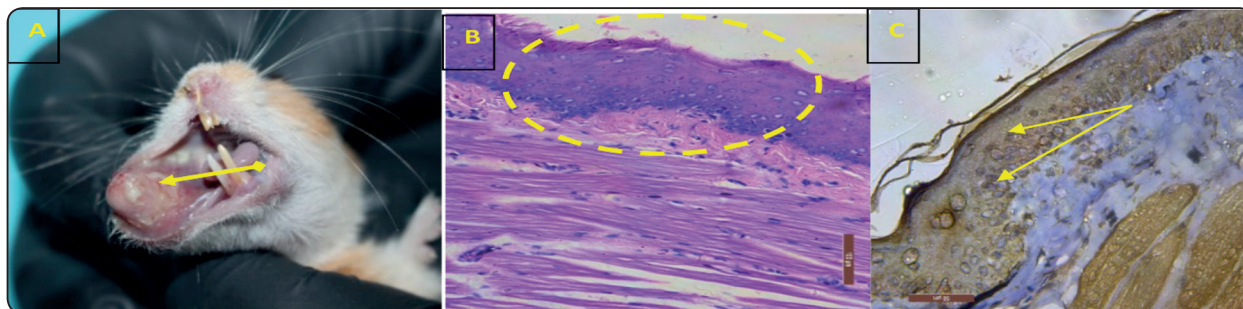


Fig. 3 A) Clinical picture of G3 the yellow arrows focused on HBP with white discoloration without ulceration and bleeding. B) H&E stained histological section of G3 the yellow dotted area focused on mild epithelial dysplasia (X 200). C) Immunohistochemical stained (Bcl-2) section of G3 the yellow arrows focused on mild (weak) positive staining which located in all epithelial layers (arrow) (X 200).

TABLE (1) Statistical analysis between normal (G 1), DMBA group (G 2) and olive oil + DMBA group (G 3) by ANOVA and Tukey's test.

	(I) Grouping	(J) Grouping	Mean Difference (I-J)	Std. Error	Sig.
Tukey's test	G1	G2	-46.74000*	.20471	.000
		G3	-8.58000*	.20471	.000
	G2	G1	46.74000*	.20471	.000
		G3	38.16000*	.20471	.000
	G3	G1	8.58000*	.20471	.000
		G2	-38.16000*	.20471	.000
ANOVA	G1		6.710	0.3624	<0.01*
	G2		53.45	0.3808	
	G3		15.29	0.1949	

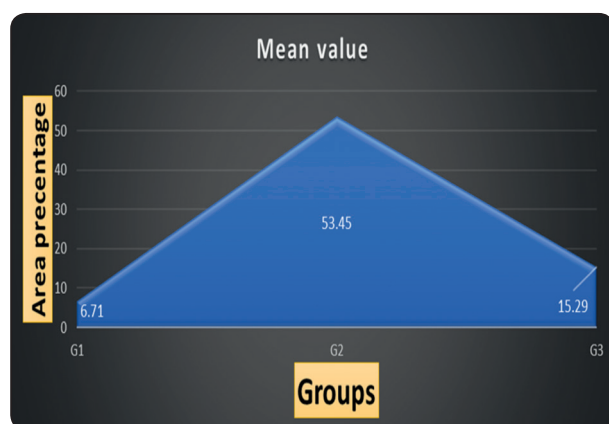


Chart 1: Mean value of area percentage of normal group (G 1), DMBA group (G 2) and olive oil + DMBA group (G 3)

## DISCUSSION

Oral epithelial dysplasia is considered as abnormal, atypical proliferation encountered fundamentally in the epithelium. Epithelial atypia, dyskeratosis and epithelial dysplasia were the terms used synonymously <sup>(21)</sup>. The term “dysplasia” is generally regarded as the perception of a disordered development. In a stratified squamous epithelium, the normal stratification and maturity affected by architectural disorder <sup>(22)</sup>. The epithelial dysplasia of the oral mucosa is believed to be accompanied by the cancer progression associated. The severe epithelial dysplasia may be progressed to malignancy in an individual lesion. However, non-dysplastic lesions may also show malignant growth <sup>(23,24)</sup>. Mainly clinical features of epithelial dysplasia appeared as a red, white or mixed lesion. There is an association between the histological severity of the lesion and the risk of malignant transformation, so the histological grading plays an important role in the transformation <sup>(25)</sup>. In the present study, all over the world olive oil is considered as one of the most common components of Mediterranean dietary fat so we used it to investigate its ability as a malignant transformation preventive and anti-carcinogenic agent. Gross observation of G3 model revealed white discoloration without ulceration and bleeding but G2 tumor mass and ulcerative lesion in mucosa. Also, in G3 reduced epithelial dysplasia incidence, three specimen with mild dysplastic while two specimen

apparently normal almost the same as G1, also there was high significant difference between (G3 and G2), where P value was ( $p \leq 0.01$ ) regarding to Bcl2 expression. The progression of tumor depends on the level of Bcl-2 which protects the cells from undergoing apoptosis <sup>(16)</sup>, our results reported the antagonist of chemopreventive substance (olive oil) with Bcl-2 expression.

This result similar to the observations of Borzì *et al* <sup>(26)</sup>, described that the olive oil contains substances which supports the chemotherapeutic possibility against colorectal cancer (CRC), these substances acting on different ways, such as oxidative damage, inflammation and even epigenetic modulation. Previous study supported that nutritional compounds play an important role in cancer initiation and development <sup>(27)</sup>, Psaltopoulou *et al* <sup>(28)</sup> revealed that, consumption of olive oil was accompanied with reduction of cancer development, managed to investigate the possible chemoprotective and anticancer effect of extra virgin olive oil (EVOO) in Mediterranean diet and this result is similar to our result in present study. Dalvi *et al* <sup>(29)</sup> who reported that the consumption of EVOO has been accompanied with diminished hazard of several cancers particularly those of upper digestive and respiratory cancers. **Fezai** *et al* <sup>(30)</sup> reported that a significant and promising inhibition of the tumor by EVOO activity in a colon cancer xenograft model assessment. It has been demonstrated that treatment of human colon adenocarcinoma cells with phenolic compounds resulted in the inhibition of all colon carcinogenetic processes such as initiation, promotion, and metastasis, triggering cell death by apoptosis <sup>(31, 32)</sup>.

The present study suggested the chemopreventive effect of olive oil against oral epithelial dysplasia. The antitumor activity seems to be related to the modulation in colonic mucosa of arachidonic acid metabolism and prostaglandin E2 synthesis,

exerted by n9 and n3 fatty acids (oleic acid and eicosapentaenoic acid respectively) present in olive oil <sup>(33)</sup>. The phenolic compounds in olive oil, besides to anti-inflammatory properties, showed also immunomodulatory effects. The immunomodulatory properties could reduce chronic inflammation in inflammatory bowel diseases (IBD) and also in other immune-mediated pathologies, such as multiple sclerosis, psoriasis, rheumatoid arthritis, systemic lupus erythematosus and inflammatory bowel diseases <sup>(34)</sup>. The primarily involved cells in the autoimmune and inflammatory responses are T lymphocytes and antigen presenting cells (APCs), which are B cells monocyte/macrophages and dendritic cells <sup>(35)</sup>. Raised levels of inflammatory cytokines (i.e., TNF- $\alpha$ , IL-8, IL-10, IL-6, IL-17) and activation of innate adaptive immune cells are involved in the pathogenesis and evolution of Inflammatory bowel diseases (IBD). On this basis, cytokine pathways modulating drugs could be used in IBD, although side effects and symptoms recurrence are common <sup>(36)</sup>. Dietary olive oil phenols seem to change clinico-pathological history in IBD, due to their anti-inflammatory properties <sup>(37)</sup>.

## RECOMMENDATIONS

After termination of the current study we recommended that the researchers must apply more research work on chemopreventive substances (Olive oil alone or combined with another chemopreventive substance) as a line of prevention of the oral cancer.

## CONCLUSION

Olive oil plays an important role in reduction and prevention of transformation of normal epithelium into epithelial dysplasia. The liquid form of olive oil may be considered as a new line of chemoprevention in oral dysplasia and the Bcl-2 expression evidenced this reduction of abnormality in epithelial dysplasia.

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