

## OXALIPLATIN ANTICANCER DRUG ACTION IN EXPERIMENTALLY INDUCED ORAL CARCINOGENESIS BY ASSESSED DNA FLOW CYTOMETRY

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### ABSTRACT

**Introduction:** Oral squamous cell carcinoma is one of the most widespread cancer which totalizes more than 90% of oral malignancies. Therefore, the conception of detaining or preventing the malignant transformation remains a viable target for future. Oxaliplatin is a third-generation platinum based chemotherapy cure that has value in the treatment against several forms of neoplasms. It forms intrastrand links between two adjoining DNA bases, hence disrupting its replication and transcription.

**Aim of the study:** The current work was carried out to report the oxaliplatin drug as a chemotherapeutic agent during DMBA-induced carcinoma in hamster buccal pouch, utilizing histopathology and flow cytometry analysis.

**Material and methods:** A total of 60 Syrian hamsters distributed as 2 animals examined for the normal pouch mucosa and 58 hamsters divided into; 6 experiments for Group I, their pouches were painted only with mineral oil. The remaining 52 animals for Group II, treated by DMBA mixed in a mineral oil. After 6 weeks, the hamsters separated into 2 subgroups; Group IIA, were persisted operated in DMBA. Group IIB, were employed to DMBA and injected with oxaliplatin.

**Results:** Oxaliplatin revealed effectiveness and tolerance in turn down the DMBA carcinogenesis procedure. Additionally, the chemotherapeutic results of oxaliplatin detected a significant reduction relation to the DNA aneuploidy and the S-phase fraction throughout the tumorigenic activity.

**Conclusion:** Oxaliplatin provided a proper strategy as a chemotherapeutic curing for control oral carcinogenesis process with a notable reduction of cancer incidence through reducing the nuclear proliferation activity and induction of cellular apoptosis.

**KEY WORDS:** Oral squamous cell carcinoma, Oxaliplatin, Flow Cytometry.

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## INTRODUCTION

Oral cancer has emerged as a deep public issue due to its relatively high incidence and mortality. Squamous cell carcinoma (SCC) is the most familiar histological type of head and neck malignancy. It is a complex and relentless cancer prone to local invasion and spreading<sup>(1)</sup>. Assimilation the molecular mechanisms demanded in the initiation and the progression of carcinomas will assist to improve its prognosis and elaboration of advanced, potent, and effectual anticancer drugs. Chemotherapy is a set of medicament which goal to stop or slow the growth of malignant cells. It is considered as a systemic remedy<sup>(2)</sup>. Oxaliplatin is a third-generation platinum based chemotherapy treatment. The task of platinum compounds is the formation of covalent adducts between platinum and some bases in the nuclear structures (about 60% of intrastrand platinum adducts are formed in the middle of 2 guanine bases and 30% are formed betwixt an adenine and a guanine bases) which guides to inhibition of nucleotides synthesis<sup>(3)</sup>. It builds DNA crosslinks with induction of a broad deformation of the genomic structure. It exerts its binding to cellular proteins and possibly interfering into RNA synthesis as well. If they are not detached from nuclear bases, oxaliplatin adducts are lethal. The cytotoxic efficacy of platinum compounds in cancer compartments can be related to suppression of DNA synthesis and its repair processes<sup>(4)</sup>.

Chromosomal aberrations are a fix mark of solid neoplasms; such cytogenetic alterations are result in a measurable deviation from DNA content of the standard cells<sup>(5)</sup>. Nuclear quota plays a sign of location in stages of the cell cycle. The normal non-dividing tissue had diploid cells, in a resting state, G<sub>0</sub> phase. As it break into the synthesis stage; DNA replication begins and in this time cells seat varying amounts of nucleic acids<sup>(6)</sup>. The response of tumors may be assisted by flow cytometric examination of nuclear bulk, that permits speedy and definitive

spotting of chromosomal variation<sup>(7)</sup>. Flow cytometry (FCM) allows a quick assessment of the ploidy status and the proliferation activity of the neoplasm by checking the chromosomal deviations and provide 2 functional points related to neoplastic progression, the ploidy state and the synthesis phase fraction (SPF). DNA ploidy is a term describes the nuclear amounts. The deviation from the regular diploid value, referred to as aneuploid; it is fully beard as an indicator of malignancy<sup>(8,9)</sup>.

Biologically and clinically pertinent hamster models are valuable tools for studying the efficiency of novel therapeutic approaches<sup>(10)</sup>. The golden Syrian hamster buccal pouch (HBP) casts of 7,12-dimethylbenz[a]anthracene (DMBA) get sequential carcinogenesis in order to research the multistep proceeding through cancer proliferation<sup>(11)</sup>. Histologically, the tumorigenic process exhibits extensive similarities to the morphology, histology, precancerous lesions and its ability to invade and metastasize to human oral SCC. In addition, expression of biochemical, molecular markers, genetic and epigenetic alterations is similar to human tissue as well<sup>(12)</sup>. The aim of the current work was to achieve the oxaliplatin force and liberality in reducing the DMBA carcinogenesis operation with reducing the proliferation and the activity of nuclear tumor quantum utilizing FCM analysis plus to the histopathology.

## MATERIAL AND METHODS

### Animals Grouping

Sixty male Syrian hamsters were secured from Theodor Bilharz Research Institute, Cairo, Egypt. Aged 6 to 8 weeks, clinically well, and heaviness about 80 to 100g. The animals were dwelt in show polypropylene cages (4 per cage) in a room had healthy temperature and humidity under 12h light/dark rotations. The hamsters were conducted at the Experimental Animal Unite, Oral and Maxillofacial Pathology Department, Faculty of Dentistry, Assiut

University. The experimental proceedings were conducted following the National Institute of Health Guide for the Care and Use of Laboratory Animals<sup>(13)</sup>. Hamsters were provided with purified soy-free food comprising 16% protein and valve water ad libitum.

The full 24 weeks of this work was designed as; a week of adaptation, after which 2 hamsters was sacrificed, after euthanized by ether inhalation. They used for histological and FCM examination of ordinary HBP mucosa. After that, the remaining 58 experiments were classified at random into 2 head groups. Group I (as control group, n=6); where the right cheek pouches of these animals were painted, 3 times a week by a heavy mineral oil only, using number 4 sable-hair brush. Group II (n=52); the HBPs were handled 3 times a week with 0.5% DMBA (Sigma, USA), dissolved in mineral oil<sup>(10)</sup>. During the carcinogenesis procedure the HBPs were observed for histopathological evaluation. At 3 and 6 weeks, 2 animals were victimized. After 6 weeks of painting DMBA (n=48); the hamsters were randomly halved into 2 subgroups. Group IIA (n=24), where the HBPs were just treated within DMBA. Group IIB (n=24); in this group, the cheek pouches painted by DMBA, and the experiments were injected intraperitoneally with oxaliplatin vials of 100mg, (Mylan, USA), as a chemotherapeutic agent. The vial was break down in 5% glucose mixture at an application of 2mg/ml. Depending on animal weight, it was administered 4mg/kg once weekly<sup>(14)</sup>. For visualization, the carcinogenesis, the HBPs were examined frequently for histological and flow cytometric evaluation. At 9, 12, 15, 18, 21 and 24 weeks; an animal was victim from Group I. Besides, 4 hamsters from each Group IIA and IIB.

### **Histopathological Evaluation**

The tested pouch of all hamster was opened longitudinally through the skin wall and examined carefully for any pathological alterations. The HBP tissues from the sacrificed animals in full groups

were processed for paraffin embedding procedure. Every tenth serial sections from every sample were stained for schedule hematoxylin and eosin (H&E), to evaluate the histopathological changes by light microscope through the research weeks. The specimens were diagnosed at Oral and Maxillofacial Pathology Department, Faculty of Dentistry, Assiut University. The identification and classification come about WHO malignant criteria<sup>(15)</sup>. Basal cell hyperplasia, dysplasia, carcinoma in situ and SCC were determined.

### **Flow Cytometry Analysis**

The specimens of the buccal mucosa of hamsters were collected for FCM estimation. At least 2 segments which had sufficient tumor fleshes (nearly 30µm thickness) from each animal were placed into labeled glass culture tubes. Samples were included for DNA-FCM investigation by a FACS Calibur Flow Cytometer (Becton Dickinson Biosciences, San Jose, California USA) at FCM Unit, Clinical Pathology Department, South Egypt Cancer Institute. Tissue fragments were submitted to mechanical disaggregation in 2mL of detergent solution (0.1ml citric acid, 0.5% Tween-20)<sup>(16)</sup>. The nuclei suspensions obtained were cleared over a 50µm nylon sieve. The staining material in this examination is The Cycle TEST™ PLUS DNA Reagent Kit (BD Biosciences). The cell cycle periods and the DNA indices of the nuclear clones were computed using the Mod-fit Software Package. The diploid figure of normal HBP was used as a reference for the identification of aneuploid clones.

### **Data Management and Statistical Analysis**

The FCM histogram analyses were declared heading to consenting basis. Tumors own a single G0/G1 peak with DNA Index (DI) of 0.95 to 1.05; to the reference sample were graded as diploid. If 2 discrete G0/G1 heights were extant, with an atypical G0/G1 peak containing a minimum of 15% of the whole events and having a corresponding G2/M

crest, then the tumors were judged as aneuploid. The DI was set down by the calculation program for DNA scanning system, as the ratio of the mean channel number of the aneuploid G0/G1 peak to the total signify channel of the G0/G1 diploid height. Therefore, lesions were assessed hypodiploid if their DI was shorter than 0.95 or hyperdiploid if their DI was more than 1.05. The SPF is the fraction of the full cell residents that are present in the S-phase of the stander cycle and is usually asserted as a ratio. The cut off for the SPF was put as the mean  $\pm 2$  standard deviation (SD) and evaluated as either being low or high. The histograms that recorded less than 5000 events showed a coefficient variation (CV); ratio of standard deviation to mean of DNA state for all nuclei in the pinnacle; higher than 10% in the G0/G1 peak, or exhibited an excessive amount of debris, and were sorted as non-evaluable<sup>(17)</sup>. The details were collected, tabulated and statistically analyzed done via computer programs (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows. The results were expressed as mean  $\pm$  standard deviation (SD). Comparison of FCM variables between the experimental groups was done utilizing Mann Whitney U test. For comparing positive data, Chi square ( $\pm 2$ ) test was performed. Exact test was used alternatively when the expected frequency is under 5. The p value less than 0.05 was appraised statistically significant.

## RESULTS

### Microscopic Evaluation

The lining epithelium of HBP mucosa had flat keratinized stratified squamous epithelium lacking rete ridges, consists of 4 distinct layers as following; a basal, spinous, thin granular, and a keratin layers (Figure 1A). The histopathological finding was evaluated as; increased number of basal cells was reviewed as an epithelium hyperplasia. Irregular epithelial stratification, unusual nuclear-

cytoplasmic proportion, high mitotic division, and loss of cellular polarity were categorized to be epithelial dysplasia. Top to bottom dysplasia, indicating carcinoma in situ. Moreover, carcinoma was identified by epithelium malignant invasion of the underlying tissues. **In Group I (control group)**, the epithelium of HBPs showed a typical appearance. Hyperkeratosis was the wholly pathological change observed in this group on the last 2 animals at 21 and 24 weeks, which developed due to continues hair brush irritation (Figure 1B).

**In Group II**, 2 out of 4 hamsters were sacrificed during the first 6 weeks of DMBA painting, showing areas of focal thickening without cellular atypia (Figure 1C). After that, the remaining experiments are divided into 2 subgroups. At 9, 12, 15, 18, 21 and 24 weeks; 4 hamsters from every group were examined for any histopathological manifestations.

**In Group IIA**, at the 9 week, the hamsters manifested epithelium hyperplasia with mild dysplasia (Figure 1D). After 12 weeks, in situ carcinoma was noted in half of the victim animals (Figure 1E). Two HBPs proved areas of micro early epithelial infiltration of the malignant cells into the underlying tissues. At the 15 week, examined pouches developed invasive, well differentiated oral SCC in 2 HBPs (Figure 1F), however, early invasion appeared in the remaining 2 experiments. By the end of the 18 week, the lining epithelium had features of well grade SCC in full hamsters. At the 21 week, the histological examination revealed well to moderate carcinoma types (Figure 1G). The remaining 4 animals from 20 to 24 weeks, presented malignant criteria such as pleomorphism, hyperchromatism, loss of cellular adhesion, and abnormal mitotic figures as a characteristic hallmark in the poorly stage of oral SCC (Figure 1H). Different grades of tumor were developed in 20 from 24 examined HBPs (83.33%). The oral lesions varied from carcinoma in situ to poorly differentiated SCC.

**In Group IIB**, the hamsters were managed by oxaliplatin after 6 weeks of DMBA painting. Throughout the first 15 weeks, no histopathological malignant changes appeared in the HBPs of most experiments. Uniquely mild epithelial dysplasia was observed in few tissues (Figure 2A). At the 18 week, an animal developed mild epithelial dysplastic, the remaining 3 HBPs signified areas of carcinoma in situ. At the 21 week, Moderate epithelial dysplastic appeared in 50% of the examined buccal pouches. Furthermore, dense inflammatory and apoptotic malignant epithelial cells were noticed (Figure 2B). The remaining sacrificed experiments, one showed some areas of early invasive SCC (Figure 2C). The other, denoted well differentiated oral SCC (Figure 2D). At the end of the 24 week, the 3

HBPs lesions sanded for well and moderate grades. No dysplastic changes were seen in the remaining hamster, loss of epithelium continuity with areas of massive necrosis and dense inflammatory reaction were noted (Figure 2E, 2F). All over the study, 8 out of 24 hamsters (23.33%) exhibited SCC which varied from early infiltration to moderate carcinoma variety. The investigation results indicating that the malignant incidence had a range of development between the experimental animals (Table 1). The difference in carcinoma induction was highly statistically significant ( $p < 0.0001$ ) when linking Group IIA and Group IIB. Moreover, the difference in cancer incidence had real statistical importance ( $p < 0.0001$ ) when versus uniting Group I and Group IIA, as well as, Group IIBB.

TABLE (1): The summary of the histopathological finding and the FCM analysis of examined HBPs in the Study.

W.	No	Animals of Study												
1 W	2	Normal HBP Mucosa (Diploid, Low SPF)												
	<b>52</b>	<b>Group II</b>												
3 W	2	No Histopathological Changes (Diploid, Low SPF)						<b>6</b>	<b>Group I</b>					
6 W	2	White Patch with Epithelial Hyperplasia (Diploid, Low SPF)												
W.	Group IIA (DMBA)				Group IIB (DMBA + Oxaliplatin)				Group I (Mineral Oil Only)					
	No	Histopath. Finding	FCM Analysis		No	Histopath. Finding	FCM Analysis		No	Histopath. Finding	FCM Analysis			
			Diploid/ Aneuploid	SPF L/H			Diploid/ Aneuploid	SPF L/H			Diploid/ Aneuploid	SPF L/H		
9 W	2	Epith. Hyperplasia	2/0	2/0	4	Epith. Hyperplasia	4/0	4/0	1	Normal Appearance	1/0	1/0		
	2	Mild Dysplasia	2/0	2/0										
12 W	2	CIS	1/1	2/0	3	Epith. Hyperplasia	3/0	2/1	1	Normal Appearance	1/0	1/0		
	2	Early Invasion	2/0	1/1		1	Mild Dysplasia	1/0					0/1	
15 W	2	Early Invasion	1/1	1/1	4	Mild Dysplasia	4/0	3/1	1	Normal Appearance	1/0	1/0		
	2	Well SCC	1/1	0/2										
18 W	4	Well SCC	1/3	1/3	1	Mild Dysplasia	1/0	0/1	1	Normal Appearance	1/0	1/0		
					3	CIS	2/1	1/2						
21 W	1	Well SCC	0/1	0/1	2	Moderate Dysplasia	2/0	1/1	1	Hyperkeratosis	1/0	1/0		
	3	Moderate SCC	1/2	1/2	1	Early Invasion	0/1	1/0						
					1	Well SCC	0/1	0/1						
24 W	2	Moderate SCC	0/2	0/2	1	sever Dysplasia	1/0	1/0	1	Hyperkeratosis	1/0	1/0		
	2	Poor SCC	0/2	0/2	2	Well SCC	1/1	2/0						
					1	Moderate SCC	0/1	0/1						
Total		20 Carcinoma	11/13	10/14		8 carcinoma	19/5	15/9						
%	24	83.33% Carcinoma	54.16% Aneuploid	58.33% H SPF	24	23.33% Carcinoma	20.83% Aneuploid	37.5% H SPF	6	No Carcinoma	Diploid	L SPF		

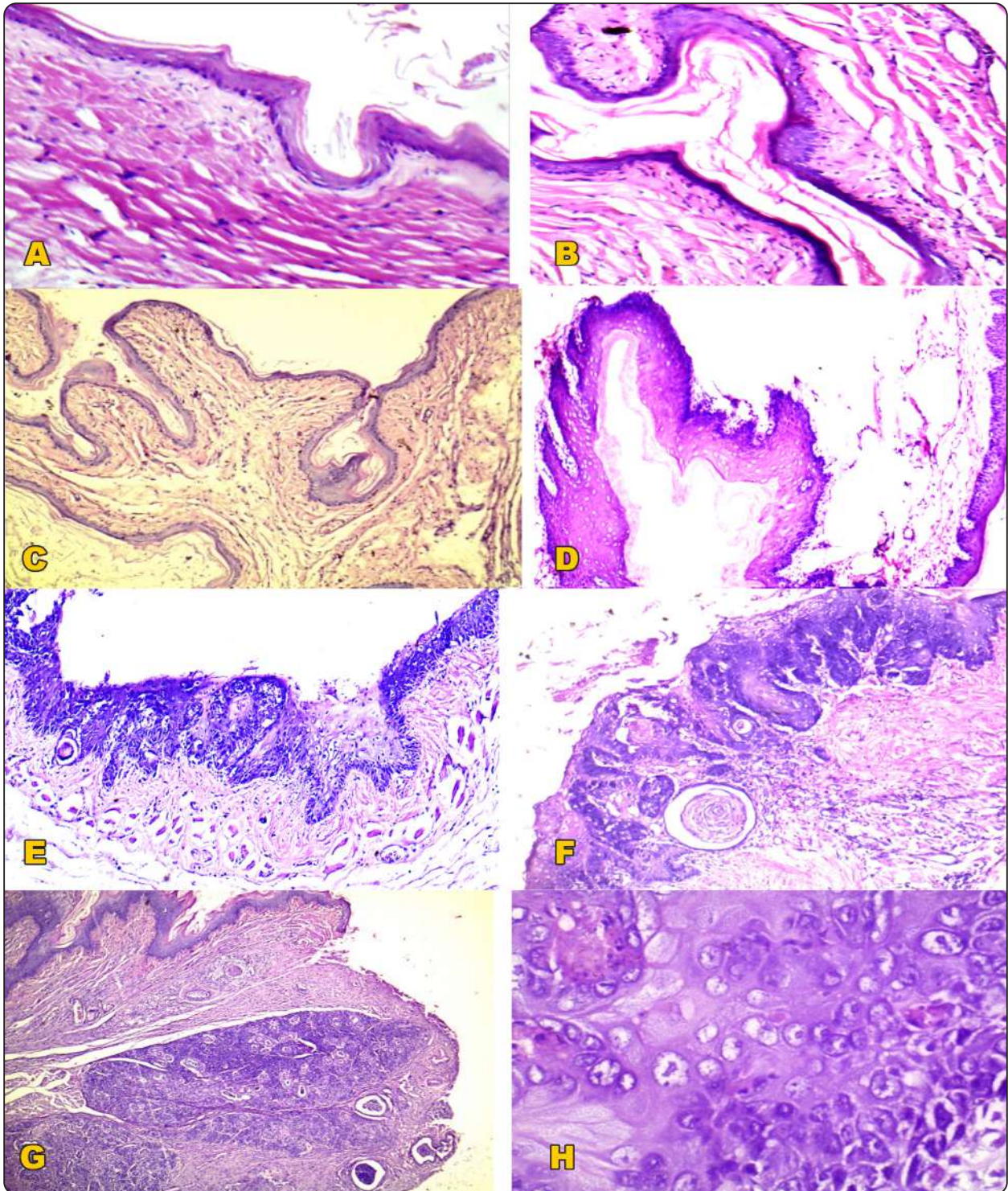


Fig. (1): A photomicrograph of Induced Carcinogenesis During DMBA Painting, Showing (A) Normal Epithelial of HBP Mucosa (H&E X100). (B) Areas of Hyperkeratosis in Epithelial Lining, Group I (H&E X100). (C) Focal Thickened Areas Missing Cellular Atypia, at 6 Weeks, Group II (H&E X100). (D) Epithelium Hyperplasia with Mild Dysplasia, at 9 Weeks, Group IIA (H&E X100). (E) Carcinoma in Situ, at 12 Weeks, Group IIA (H&E X40). (F) Well Differentiated Oral SCC, at 15 Weeks, Group IIA (H&E X40). (G) Moderate Type SCC in the Form of Malignant Cell Nests, at 21 Weeks, Group IIA (H&E X40). (H) Poorly Stage of Oral SCC with Evident Malignant Criteria at 24 Weeks, Group IIA (H&E X40).

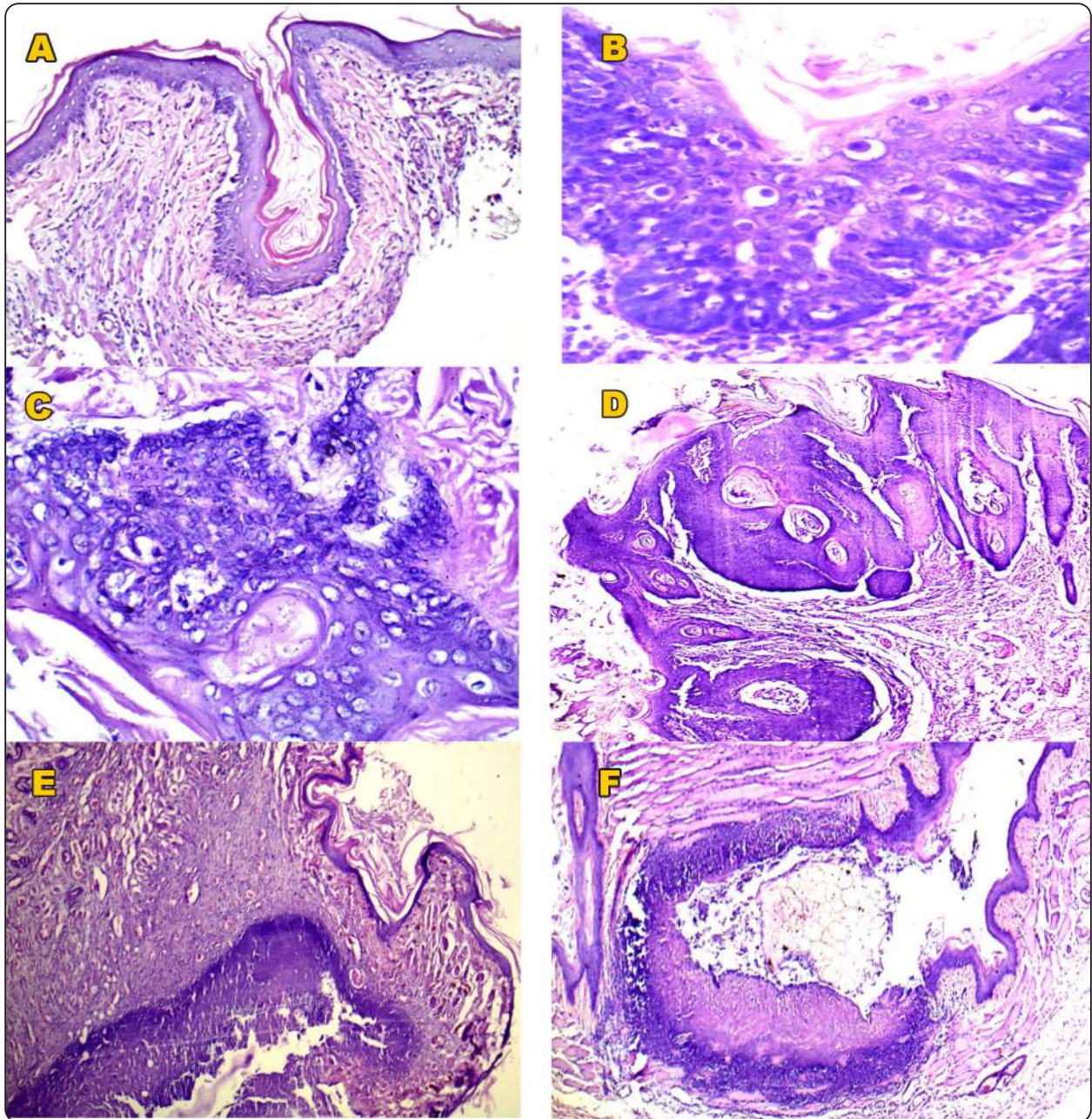


Fig. (2): A photomicrograph in Group IIB, Showing (A) Mild Epithelial Dysplasia, at 12 Weeks (H&E X100). (B) Evident Apoptotic Cell Activity, at 16 Weeks (H&E X400) (C) Area of Early Invasive Oral SCC, at 21 Weeks (H&E X400). (D) Well Differentiated Oral SCC, at 21 Weeks (H&E X100). (E, F) Massive Areas of Necrosis and Dense Inflammatory Reaction, at 24 Weeks (H&E X100).

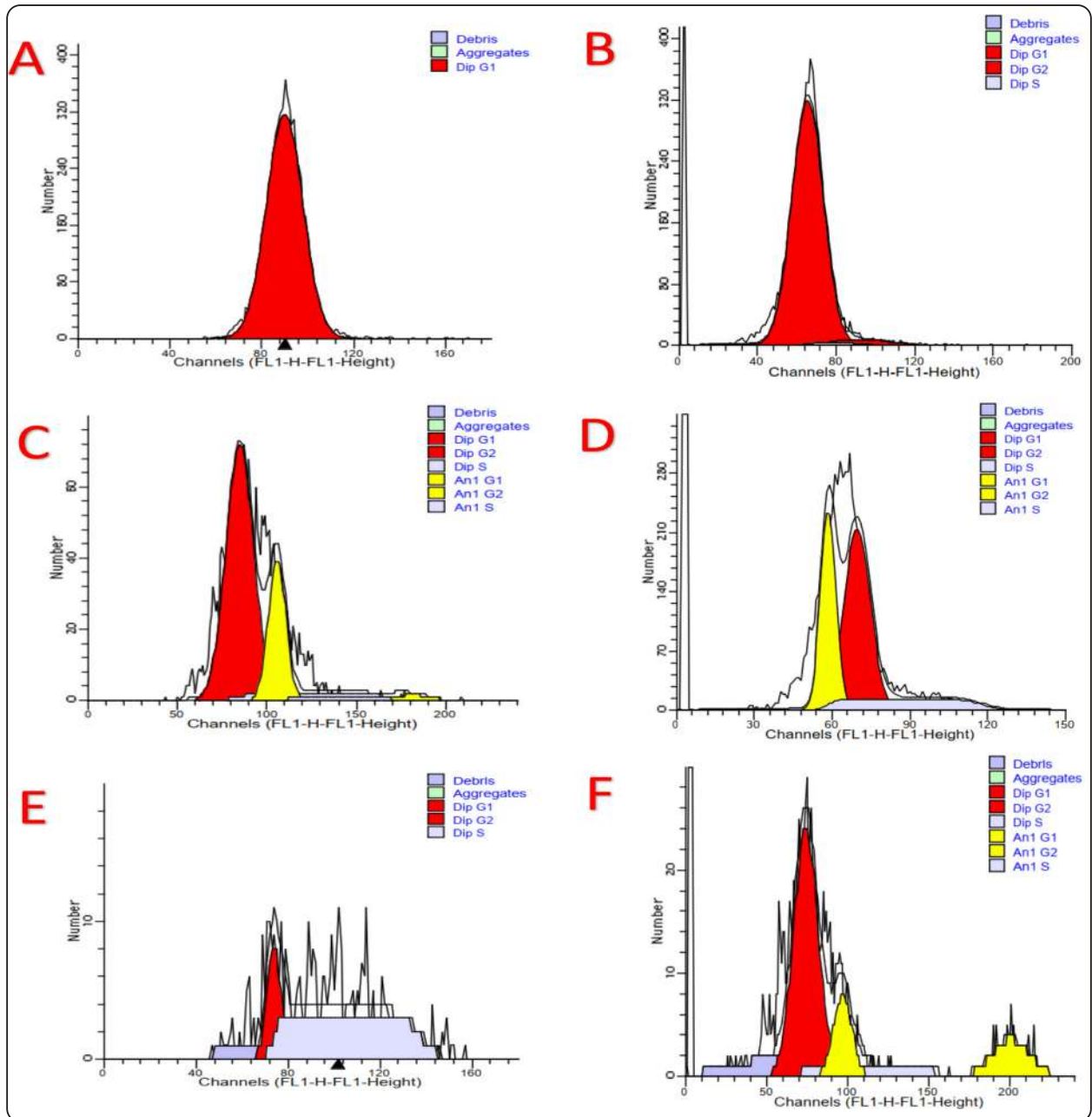


Fig. (3): DNA Frequency Histograms Showing (A) Single G0/G1 Diploid Peak of Normal Oral Mucosa. (B) Single Diploid Peak in Group I, with Small Numbers of Cells in SPF (1.7%). (C) Aneuploid Malignant Tumor in Group IIA Showing Hyperdiploid (DI=1.25) and Low SPF (7.53%). (D) Aneuploid Lesion in Group IIB Showing Hypodiploid (DI=0.84) and high SPF (31.24%). (E) Diploid Peak in Group IIA Showing High SPF (77.88%). (F) Aneuploid Neoplasm in Group IIB Showing Low SPF (18.49%).



### Flow Cytometric Review

A number of 60 HBPs, were analyzed by FCM. The single peak of conventional pouch oral mucosa, was considered the standard reference G0/G1 (Figure 3A). Total hamsters were set off with paraffin oil in Group I, were diploid and small numbers of cells in the SPF (Figure 3B). After malignancy proliferation by DMBA, 13 from 24 animals (54.16%) in Group IIA showed considerable variation in aneuploid DNA content (Figure 3C). The aneuploid HBP lesions decreased in Group IIB, as the oxaliplatin was injected. Over the research, 5 of 24 hamsters (20.83%) had aneuploid nuclear pattern (Figure 3D). The difference in ploidy state between Group I and Group IIA or Group IIB and connecting Group IIA and Group IIB tumors was statistically highly significant ( $p= 0.0001$ ). The aneuploid malignant tissues were either hyperdiploid or hypodiploid. In hyperdiploid lesions, 8 in Group IIA to 2 in Group IIB; DI ranged from 1.05 to 1.76 within a mean of 1.30. Whereas, in hypodiploid cases, 5 in Group IIA Group IIA to 3 in Group IIB; DI ranged from 0.47 to 0.98 with a signify of 0.62. No important difference ( $p= 0.463$ ) in number of hyperdiploid and hypodiploid state to linking Group IIA and Group IIB.

The calculated SPF values for the control animals, Group I, was very low which ranged from 0% to 1.88%, within a mean of 1.07%. After carcinoma induction by DMBA, the SPF values raised remarkably ( $p=0.001$ ), in Group IIA, to reach up to 80.42% within a signify of 38.14% in 14 out of 24 hamsters (58.33%) (Figure 3E). Meanwhile, oxaliplatin therapy actually ( $p= 0.001$ ) reduced the number of cases having high SPF, where 37.50% (9/24) of experiments had high SPF values; 7.45% and 27.19% with a mean of 19.52% (Figure 3F).

### DISCUSSION

Oral neoplasms are often preceded by a premalignant step accessible to visual inspection and opportunities for earlier detection to reduce morbidity and mortality<sup>(18)</sup>. Superior understanding of the aetiopathogenesis should lead to more accurate and active therapeutics. Curing is aided by detection of cellular and molecular deviations<sup>(19)</sup>. It was proved that the oxaliplatin is the most active drugs for care of colorectal cancer, especially its metastatic form<sup>(20)</sup>. Moreover, Pages et al. suggested that its chemotherapeutic role was safe and effective<sup>(21)</sup>. In addition, De Felice et al. evidenced that oxaliplatin added crucial results on distant metastasis control in locally advanced rectal tumors as well<sup>(22)</sup>. Its modified products allowed their use in several kinds of neoplasms. It was tested for treating esophageal, biliary tract, pancreatic, gastric and hepatocellular cancers<sup>(23-25)</sup>. Furthermore, Wang group detailed that a novel oxaliplatin derivative had a promising anticancer effects in multiple malignant cell lines<sup>(26)</sup>. Meanwhile, adenoid cystic carcinoma of the salivary glands confirmed an objective response to oxaliplatin<sup>(27)</sup>. In contrast, some lesions had a platinum chemotherapy resistance as the epithelial ovarian tissues which demonstrated poor outcome results<sup>(28)</sup>. Further support can be derived from Liu et al. that reported a platinum sensitivity in human lung and ovarian cancer cells<sup>(29)</sup>. The causes for different oxaliplatin efficacies were not well understood but the individual tumor characteristics might determine the treatment efficacy, because the DNA structures was pondered the preferential cytotoxic target.

In the present experimentation, oxaliplatin validated consequences and magnanimity in reducing the DMBA malignancy action. In agreement to the results of the present work, Li et al. indicated that oxaliplatin can inhibits development of oral SCC<sup>(30)</sup>. Additionally, Nishida et al. point to a strong anti-tumor power of the drug in advanced esophageal SCC<sup>(31)</sup>. These results did not differ much from

other studies done by Sun et al. and Lo et al.<sup>(32, 33)</sup>. This goes with the results of Hussein et al. which concluded that oxaliplatin provides a curing role through the operation of oral carcinogenesis and may be employed as chemotherapeutic agent for carcinomas<sup>(34)</sup>. Further, Xu et al. evinced that the oxaliplatin raised the apoptotic rate of human SCC, that designated a new target for the healing of oral neoplasms<sup>(35)</sup>. This observation is in deal with the present search which detected apoptotic cells activity within lesions tissues. The therapeutic efficacy of such platinum-based drug is believed to, at least in part, result from formation of platinum-DNA adducts, followed by nuclear damage response and ultimately apoptosis<sup>(36)</sup>. Over and above, Shen et al. tolled that oxaliplatin was a promising agent for chemotherapy in treating esophageal SCC<sup>(37)</sup>. Also, separated studies recommended that regimen was a treatment option for metastatic head and neck SCC<sup>(38, 39)</sup>. Opposite, Lim et al. hinted that the oxaliplatin did not lead to better efficacy in node-positive esophageal SCC patients<sup>(40)</sup>. A possible explanation for this negative result could be that over half of the enrolled patients in the study had advanced nodal diseases. So that, the cure by chemotherapy alone was not probably sufficient to control the recurrence. As well, Fakhrian and colleagues supported the poorer oxaliplatin outcomes in esophageal SCC patients when compared to other platinum adduct as cisplatin<sup>(41)</sup>. This announced that early curing gave more marked results than when administrated in advanced stage of developing carcinoma. Further support can be derived from the assay of Yang et al. which resulted that the time factor should be inspected when treating the oral SCC patients with oxaliplatin in order to attain a better efficacy, reduce the adverse reactions and improve the survival time<sup>(42)</sup>. Besides, the interaction of the different medication made down regulation for the proper working of the platinum adducts<sup>(43)</sup>.

In the current article, a significant differences recorded in both ploidy state and SPF value in the tested hamsters between DMBA group (Group

IIA) and DMBA + oxaliplatin group (Group IIB). This supports the anticancer role of oxaliplatin during DMBA induced carcinoma. The results are comparable to the concept of using the nuclear morphometric aspects and ploidy state by FCM as prognostic markers of malignancy<sup>(44, 45)</sup>. Normally, DNA damage is sufficient to slow transit S-phase or cause a block in G2 to allow correct of potentially lethal damage<sup>(46)</sup>. Along with oxaliplatin modulate the cell cycle through intrastrand links in the middle of 2 adjacent DNA bases. This modulation reduces the aneuploidy and the SPF activity during the course of treatment, which depends on the tumor type and is drug concentration specific<sup>(47)</sup>. Another paper corroborated that oxaliplatin may induce cell death through arrest ribosome biogenesis<sup>(48)</sup>. Compelling evidence has shown that toxicity of platinum-DNA adduct is associated into free radical generation, nucleic acids impairment, endocrine and mitochondrial dysfunctions, oxidative inflammation, apoptosis, endoplasmic reticulum stress, activation of regulator signaling proteins, and cell cycle arrest<sup>(49)</sup>. Antithesis, Saintas and colleagues found that the formation of acquired oxaliplatin resistance is a major reason for the failure of anticancer therapies success after initial response<sup>(50)</sup>. Plus that the genomic instability may favor the generation of more aggressive tumor cells with a reduced propensity for undergoing apoptosis and developed selective chemotherapy resistance<sup>(51)</sup>. Moreover, Guo et al observed that aneuploidy status in malignant cells; partially associated with the acquired drug resistance<sup>(52)</sup>.

## CONCLUSION

Oxaliplatin had great repression rates of proliferation and migration of tumorigenesis activity during DMBA carcinogenesis process. Future research is required to prove developed early detection methods for cancer will be an aid in the accurate and proper systemic treatment of the neoplasms. It could provide serious improvements in the survival of malignant patients.

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