MODULATORY EFFECT OF MUSHROOM EXTRACT ON 7, 12-DIMETHYLE ANTHRACENE (DMBA)-INDUCED LIP CANCER IN ALBINO RATS

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ABSTRACT

Background: Oral cancer constitutes approximately 25% of all cancer types. 7, 12-dimethylbenz (a) anthracene (DMBA) has been widely used as an active chemical inducer for mammary carcinogenesis. COX-2 enzyme is frequently found in certain types of cancer, including colon, lung, breast, pancreas, head, and neck cancers and is usually associated with poor prognosis and short survival. Mushrooms contain polysaccharides that are thought to inhibit tumor growth and viral infection by stimulating immune cells.

Objective: The present work aimed to investigate the effect of mushroom extract on DMBA-induced lip carcinogenesis in albino rats and correlate the results with COX-2 tissue expression.

Materials and methods: Thirty-six adult female albino ratswere divided into three groups as follows (twelve rats each); *Group 1:* (–ve control group in which each rat was given 1 ml sesame oil via gastric gavage as a single weekly dose for 6 weeks). *Group 2:* DMBA (+ve control group in which each rat was given DMBA emulsion via gastric gavage as a single weekly dose for 6 weeks). *Group 3:* DMBA+M (Experimental group in which each rat was given DMBA emulsion for 3 weeks and was co-administered with mushroom extract (dissolved in distilled water) for the following 3 weeks via gastric gavage. Lip specimens were processed for hematoxylin and eosin staining and immunohistochemically prepared for COX-2 expression. The area fraction expressed by COX-2 was calculated histomorphometrically. The data was expressed statistically as mean ± standard deviation (S.D.).

Results: Both mucous membrane and skin side of *Group 2* specimens showed signs of basement membrane rupture as well as hyperchromatic, pleomorphic and dysplastic epithelial cells. Connective tissue showed invasionwith dysplastic epithelial cells, dilated and congested blood vessels with many inflammatory cells. However, the mucous membrane side *Group 3* exhibited almost intact basement membrane and only few hyperchromatic epithelial cells. The connective tissue displayed less inflammatory cell infiltration with almost absent invasion while, skin side of *Group 3* presented some epithelial areas with intracellular edema but apparently minimal invasion of epithelial cells in the dermal connective tissue.

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The immunoexpression pattern of COX-2 in both mucous membrane and skin side of *Group 2* was clear and strongly positive in epithelial and connective tissue cells. However, *Group 3* showed down regulated expression of COX-2.Statistical results presented a significantly reduced expression of COX-2 in *Group 1* followed by *Group 3*, while the highest expression was shown in *Group 2*.

Conclusion: Mushroom extract is a possible natural source that may limit the carcinogenic effect of a known carcinogenic agent by suppressingits inflammatory reaction and invasive power.

Keywords:DMBA, mushroom extract, lip cancer, COX-2

INTRODUCTION

Oral cancer is a common neoplasm worldwide, particularly in the developing countries as in India, Srilanka, Vietnam and Yemen. It constitutesapproximately 25% of all cancer types[1]. Recently, the incidence of oral cancer and mortality rate have been increased in the US, Japan, Germany and Scotland.[2, 3]

The survival rate of oral cancer patients has not significantly improved despitethe recent advances in radiotherapy and chemotherapy[4].Among the risk factors, tobacco and alcohol are the major ones in development of oral cancer [5].The development of oral cancer is a multistep process requiring initiation, promotion and progression.[6,7]

Malignant tumors as mammary tumors may arise spontaneously in few animal species, for example dogs, rats, and mice [8]. For research work, most of the studies about experimental breast and oral carcinogenesis are conducted on rodents. [9]

Cancer chemoprevention is a new promising strategy for prevention, inhibition or reversion of carcinogenesis, induced by specific natural or synthetic chemicals.

The most widely usedactive chemical inductors of mammary carcinogenesis are 7, 12-dimethylbenz(a) anthracene (DMBA) and N-methylnitrosurea[10, 11]. (DMBA) induced oral carcinogenesis in golden Syrian hamsters is an accepted and well recognized experimental model for studying biochemical, histopathological and molecular alterations occurring in oral carcinogenesis. DMBA induced molecular changes in the buccal mucosa of golden Syrian hamsters closely mimics or resembles that of human oral tumors.[12, 13]

DMBA is highly lipophilic and requires metabolic activation for its carcinogenicity. Several tissues can activate DMBA, and these include the mammary glands. In the breast, DMBA is converted to epoxides, active metabolites with a capacity for damaging the DNA molecule, the main event in initiation of carcinogenesis.[14]

DMBA was also topically applied to the cheek pouch of the Syrian golden hamster and was found to producesquamous cell carcinoma.[15]

In recent years, there is an increasing evidence for a correlation between high consumption of fruits and vegetables and the reduced risk of cancer. Thus, suggesting that natural products may offer a possible protective effect against many types of cancer, including oral cancer.[16, 17]

Mushrooms have anti-cancer, anti-inflammatory and immune-supporting properties. Mushrooms contain polysaccharides that are thought to inhibit tumor growth and viral infection by stimulating immune cells. They have also been shown to trigger programmed cell death in breast cancer cells. In case control studies, consuming mushrooms regularly has been associated with decreased risk of breast cancer in both pre- and postmenopausal women.[18]

About 100 mushroom species were being studied for their health-promoting benefits, and about a half dozen actually stand out for their ability to deliver a tremendous boost to the immune system. In fact,

some of the most potent immunosupportive agents come from mushrooms, and this is one reason why they're so beneficial for both preventing and treating cancer.

Long-chain polysaccharides, particularly alpha- and beta-glucan molecules, are primarily responsible for the mushroom's beneficial effect on human immune system. In one study, adding one or two servings of dried shiitake mushroom was found to have a beneficial, modulating effect on function of the immune system.[19]

Other naturally-occurring compounds in mushrooms such as fungal proteins, lectins, peptides and laccases have also been reported to have significant effects on immune function. A protein-bound polysaccharide extract from turkey tail mushroom is also being used to boost cancer patients' immune function in countries including Japan.[20]

Compounds in Shiitake and Maitake mushroom have been shown to trigger programmed cell death in breast cancer cells.[18]

From superficial to deep, the layers of the upper and lower lips include the epidermis, subcutaneous tissue, orbicularis oris muscle fibers, and mucosa. In cross-section, the superior and inferior labial arteries can be observed as they course between the orbicularis muscle fibers and the mucosa, which is thick (1500μm), non keratinized and firmly attached to the underlying muscle. The vermilion border (only in humans) is composed of thin keratinized stratified squamous epithelium that covers numerous capillaries, which give the vermilion its characteristic color. Numerous minor salivary glands can be observed in a histologic section of the lip. Hair follicles and sebaceous glands, with sweat glands in between, are located throughout the skin side of the lip; however, these structures are absent in the vermilion border. It is covered by keratinized epithelium of moderate thickness and a rather thick keratin layer. [21]

Investigation of biomarkers' presence, quantity and expression pattern would help to correlate to the probability of malignant transformation of a cell or tissue. Evaluation of biomarkers would also help to identify patients who require more aggressive management.[22]Molecular markers of cell proliferation, apoptosis, angiogenesis and inflammation have been studied as potential tools to predict the prognosis of patients with oral squamous cell carcinoma.[23, 24]

Two COX enzymes: COX-1[25] and COX-2 [26] were identified. In human, COX- 1 is found to be expressed in a wide range of tissues including the lungs, kidneys, stomach, small intestine and colon. Thus, COX-1 is considered a housekeeping enzyme responsible for maintaining basal prostaglandin levels, important for tissue homeostasis. In contrast, most tissues do not normally express COX-2 under normal stress conditions, exceptions include the central nervous system[27] and seminal vesicles. [29]

Overexpression of COX-2 (or exogenous PGE2) was found to increase migration and intercellular adhesion molecule 1 (ICAM)-1 expression in human oral cancer cells.Intercellular adhesion molecule-1 (ICAM-1, also called CD-54), a member of the immunoglobulin family, is a surface glycoprotein that mediates adhesion-dependent cell-to-cell interactions [30, 31]. The extracellular domain of ICAM-1 is essential for the trans-endothelial migration of leukocytes from the capillary bed into the tissue[32]. ICAM-1 was also found to facilitate the movement of cells through the extracellular matrix[32] and play an important role in lung cancer cell invasion[33]. ICAM-1 antibody hasbeen correlated to the invasiveness of breast cancercells [34]. Therefore, ICAM-1 may play a critical role in tumor genesis,and its inhibition may prevent metastasis.

COX-2 is also frequently found in other types of cancer, including colon, lung, breast, pancreas, head, and neck cancers [35-37]and is usually associated with poor prognosis and short survival.

The purpose of our study is to investigate the effect of mushroom extractson DMBA-induced lip carcinogenesis in albino rats and correlate the results with COX-2 tissue expression.

Methodology:

Animals and Materials:

The whole experiment was carried out at the animal house of Faculty of Medicine, Cairo University.All steps of the experiment were approved by the Ethics Committee of Experimentation on Animals of Faculty of Medicine, Cairo University. Thirty-six adult female albino rats weighing 180-200gm were used in this study.

7, 12- dimethylbenz (a) anthracene (DMBA) was purchased from Sigma Aldrich chemical company. It was supplied in the powderform and dissolved in sesame oil to form an emulsion. Cancer was induced by a single weekly intragastricadministration of DMBA (65 mg/kg of body weight) in 1.0 ml of sesame oil.[38]

Mushroom extract was supplied in the form of tablets and purchased from Source Naturals, INC., USA. as a mixture of (Shiitake, Maitake, Reishi and Turkey Tail Mycelia).The tablets were ground to a powder formand administered by dose of 200mg/kg body weight, dissolved in an inert solvent (distilled water).[39]

Experimental design:

The rats were divided into three groups as follows (twelve rats each):

Group 1: Control (–ve control group in whicheach ratwas given 1 ml sesame oil viagastric gavage as a single weekly dose for 6 weeks).

Group 2: DMBA (+ve control group in which each ratwas givenDMBA emulsion via gastric gavage as a single weekly dose for 6 weeks).

Group 3: DMBA+M (Experimental group in which each rat was given DMBA emulsionfor 3 weeksco-administered with the mushroom extract (dissolved in distilled water) for the following 3 weeks viagastric gavage.

The animals were sacrificed by cervical dislocation. Biochemical studies were conducted on lip mucous membrane side and skin side of control and experimental animals, in each group. For histopathologic examination, lip tissues were dissected out and fixed in 10% phosphate buffered formalin for 48 hrs. The specimens were then washed under tap water, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin wax. Sections of 4-5μm in thickness were cut in a rotary microtome and mounted on clean glass slides. Tissue sections were then deparaffinized in xylene and rehydrated in descending ethanol series ending with pure H2O (Millipore Corporation, Temecula, CA, USA).The sections were then ready to be stained with H&E solutions (Sigma, St. Louis, MO, USA), to verify histological details.[40]

Immunohistochemical staining:

Paraffin embedded tissue sections were dewaxed and rehydrated through grade ethanol to distilled water. Endogenous peroxidase was blocked by incubation with 3% H_2O_2 in methanol for 10 minutes. The antigen retrieval was achieved by microwave in citrate buffer solution (2.1 g citric acid/L D.H2O; 0.37g EDTA/L D.H2O; 0.2g Trypsin) (pH 6.0) for 10 minutes, followed by washing step with Tris-buffered saline (8g NaCl; 0.605g Tris) (pH 7.6). The tissue section was then incubated with power BlockTM reagent (BioGenex, San Ramon, CA, USA), universal proteinaceous blocking reagent, for 15 minutes at room temperature to block non-specific binding sites. The tissue sections were then incubated with the primary antibody (COX-2- Dako, Carprinteria, CA, USA) overnight at 4°C. The bound primary antibody was detected by incubation with the secondary antibody conjugated with horseradish peroxidase (BioGenex, San Ramon, CA, USA) for 30 minutes at room temperature.

After rinsing with Tris-buffered saline, the antigen-antibody complex was detected using 3, 3′-diamminobenzidine, the substrate of horseradish peroxidase. When acceptable color intensity was reached, the slides were washed, counter stained with hematoxylin and covered with a mounting medium. Each slide was microscopically analyzed and enumerated the percentage of the positively stained cells semi-quantitatively. The percentage of positive cells was scored according to the degree of staining.[41]

Statistical analysis

The area fraction expressed by COX-2was calculated histomorphometrically. The datawere expressed as mean ± standard deviation (S.D.) Numerical data were explored for normality by checking the data distribution, calculating the mean and median values, evaluating histograms and normality curves and using Kolmogorov-Smirnov and Shapiro-Wilk tests. Independent t test was used for comparison between groups. The significance level was set at $P \leq 0.05$.

RESULTS

Examination of H & E stained sections of mucous membrane side of the negative control group**(Group 1)** revealed almost normal histological features regarding the surface epithelium and the underlying lamina propria.The epithelium consists of stratum basale resting on intact basement membrane followed by stratum spinosum, stratum granulosum and stratum corneum. Basal layer consists of short columnar cells with a centrally located nucleus. Stratum spinosum showed well defined polyhedral cells with a centrally located nucleus. Stratum granulosum was apparently thin, their cells were flat with centrally located flattened nuclei. Keratohyaline granules could be detected in this layer. Stratum corneum showed apparently thin keratin layer. In the lamina propria, densely packed collagen fiber bundles with few

detected inflammatory cells scattered throughout the connective tissue (C.T) could be noticed (fig. 1 a). Also, the skin side of specimens from the same group showed almost normal keratinized stratified squamous epithelium of the epidermis with intact basement membrane. The cross sections of hair follicles appeared normal. The connective tissue of the dermis showed minimalinflammatory cellinfiltration (fig. 2 a).

Examination of H & Estained sections of mucous membrane side of **Group 2** revealedsigns of rupture of basement membrane in several areas. Moreover, the epithelium showed hyperchromatic and pleomorphic dysplastic basal cells' nuclei, hyperchromatic dysplastic cells in the granular cell layer and disrupted keratin layer. Connective tissue examination revealed its invasion by dysplastic epithelial cells with obviously dilated and congested blood vessels surrounded by inflammatory cells (fig. 1 b).

Skin side of the same group, demonstrated epidermal cells with hyper chromatic, karyolitic and degenerated nuclei. Uneven epithelial thickness in some areas was noticed. Other disrupted areas with increased keratin thickness were also noticed. Hair follicles showed areas of intra and intercellular edema of the associated sebaceous gland cells with disrupted hair shafts. Dermal invasion by epithelial cells was observed with massive infiltration of inflammatory cells. Wide areas of complete connective tissue loss were also observed (fig. 2 b).

Histopathological inspection of **Group 3** exhibited almost intact basement membrane, only few cells were hyperchromatic. The connective tissue displayed less inflammatory cell infiltration compared to group 2with almost absent invasion by epithelial cells (fig. 1 c). Skin side presented areas of intracellular edema and minimalinvasion of epithelial cells in the dermal connective tissue. Less inflammatory cell infiltration was detected in comparison to group 2 (fig. 2 c).
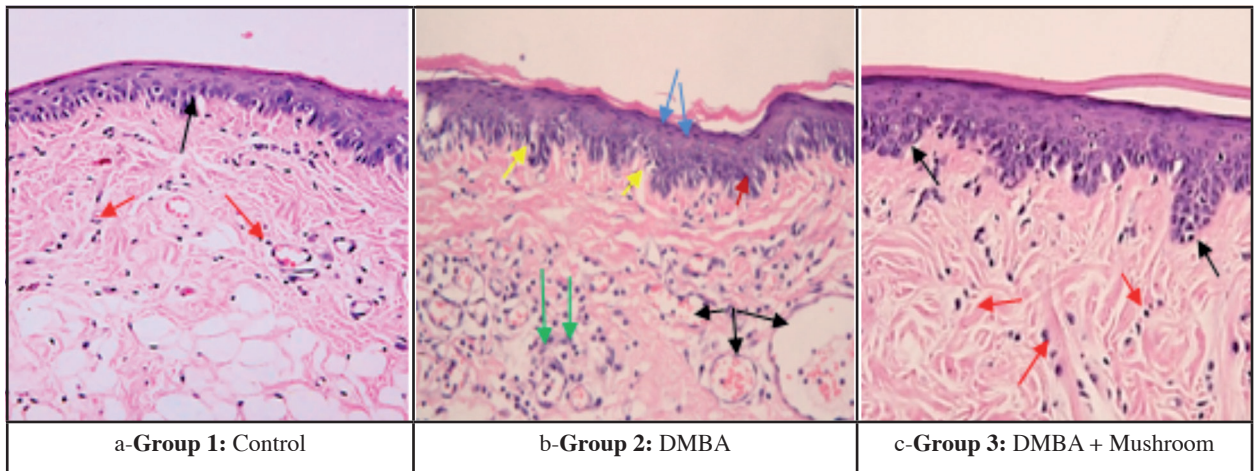
| a-**Group 1:** Control | b-**Group 2:** DMBA | c-**Group 3:** DMBA + Mushroom |

Fig. (1) Photomicrograph ofH & E stained sections showing: a- Group 1(-vecontrol group), almost normal stratified squamous epithelium (black arrow) and lamina propria with minimal inflammatory cells(red arrows). b- Group 2(DMBA / +ve control), showing raptured basement membrane(yellow arrow), hyperchromatic and pleomorphic dysplastic basal cells (red arrow), hyperchromatic dysplastic epithelial cells in the granular cell layer (blue arrows), dysplastic epithelial cells in the connective tissue (green arrows), dilated, congested blood vessels and inflammatory cells (black arrows). c- Group 3(DMBA + M),almost intact basement membrane (black arrows), inflammatory cells in the connective tissue (red arrows). (org. mag. X 400)
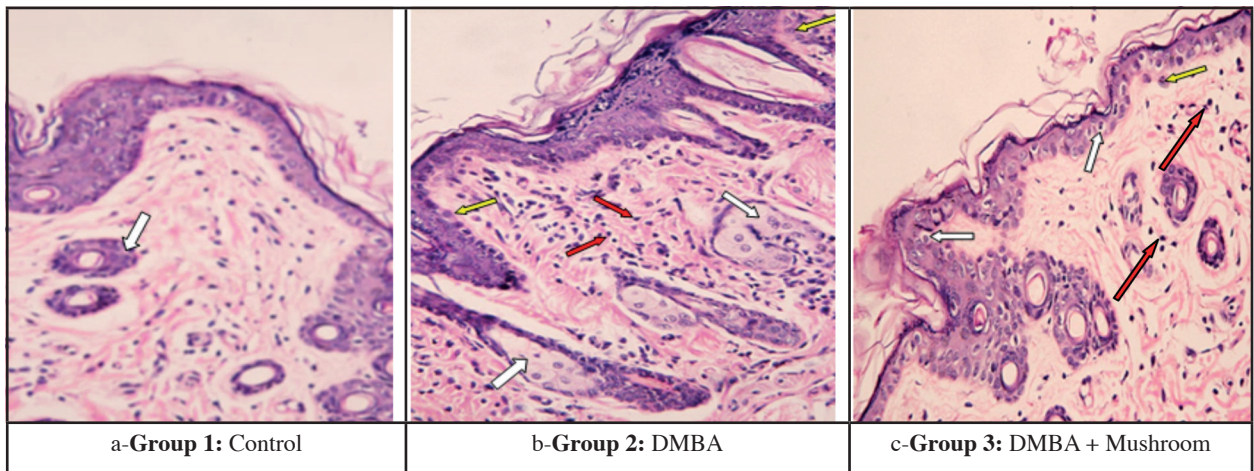

| a-**Group 1:** Control | b-**Group 2:** DMBA | c-**Group 3:** DMBA + Mushroom |

Fig. (2) Photomicrograph of specimens stained with H&E (skin side of lip):a-Group 1 (-ve control group),showing almost normal histological features of skin epidermis and hair follicles (white arrow).b- Group 2(DMBA / +ve control), showed disrupted epithelial layer with uneven thickness, the CT showed obvious inflammatory cells infiltration (red arrows) and invasion by epithelial cells (yellow arrows). Cellular edema in sebaceous glands sections (white arrows).c- Group 3(DMBA + M), epithelial cellswith areas of intracellular edema (white arrows).Very few epithelial cells appeared in the dermal connective tissue(yellow arrow). Less inflammatory cell infiltration(red arrows). (org. mag.X 400)

The immunoexpression pattern of COX-2 in labial mucosa of all groups was shown in (fig. 3). **Group 1** showed normal labial mucosa with minimal detectable expression of COX-2 (fig. 3 a). On the other hand, **Group 2** presented clear strong positive nuclear and cytoplasmic expression in both epithelial and connective tissue cells (fig. 3 b). However, **Group 3** showed down regulated expression of COX-2 in the labial mucosa of the rats treated with DMBA and mushroom extract (fig. 3 c).

The immunoexpression pattern of COX-2 in skin side of the lip of all groups was shown in (fig. 4). **Group 1** showed normal skin with slight detectable expression of COX-2 (fig. 4 a). On the other hand, **Group 2** presented well defined positive nuclear and cytoplasmic expression in both epithelial and connective tissue cells (fig. 4 b). However, **Group 3** showed down regulated expression of COX-2 in the skin sideof the rats lips treated with DMBA and mushroom extract (fig. 4 c).
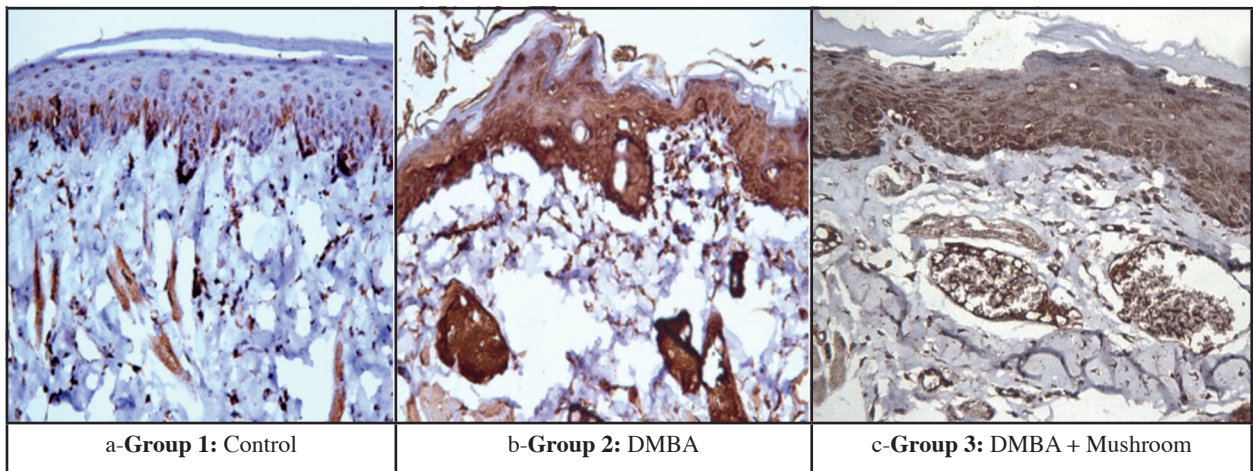

| a-**Group 1:** Control | b-**Group 2:** DMBA | c-**Group 3:** DMBA + Mushroom |

Fig. (3) Photomicrograph showing the immunoexpression pattern of COX-2 in labial mucosa of a-Group 1 (-vecontrol group) with minimal expression. b-Group 2(DMBA +ve control) strong positive nuclear and cytoplasmic expression. c-Group 3 (DMBA + M)moderate expression.(org. mag. X400)
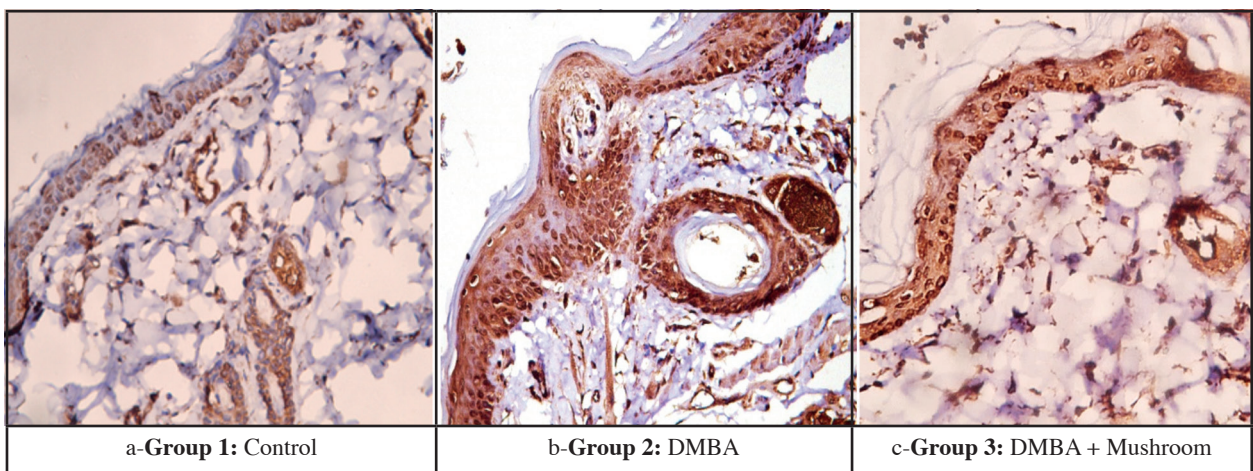

| a-**Group 1:** Control | b-**Group 2:** DMBA | c-**Group 3:** DMBA + Mushroom |

Fig. (4) Photomicrograph showing the immunoexpression pattern of COX-2 in lip skin side of a- Group 1(-vecontrol group) slight detectable expression.b-Group 2 (DMBA) well defined positive reaction.c-Group 3 (DMBA + M) moderate reaction. (org. mag. X400)

Statistical findings indicated a significantly reduced expression of COX-2 in group 3 than in group 2. By calculating the area fraction, the least expression was measured in group 1 followed by group 3. The highest expression was measured in group 2.

The mean and standard deviation of all three groups are presented in (table 1). The histogram of the collected data for the group treated with DMBA alone (group 2) and the group treated with DMBA and Mushroom extract (group 3) respectively were shown in (fig. 5).

Table (1) The mean and standard deviation of the area fraction of the three groups (Group 1: control group, Group 2: DMBA, Group 3: DMBA with Mushroom extract).

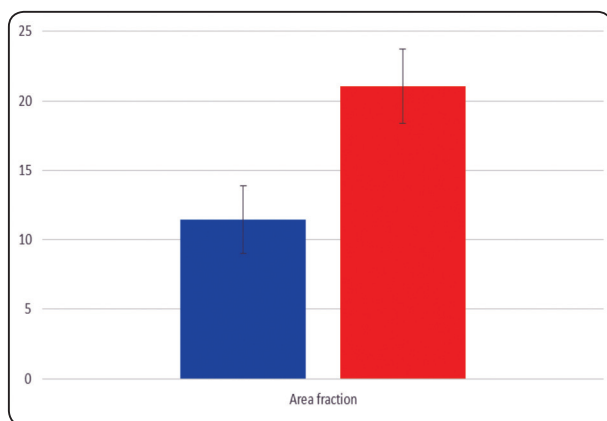|	Group 1		Group 2		Group 3		P value
	Mean	Std. deviation	Mean	Std. deviation	Mean	Std. deviation	
Area fraction	7.98	1.87	21.04	2.69	11.48	2.46	<0.001

Fig. (5) Histogram representing the collected data for the group treated with DMBA only (group 2) and the group treated with DMBA and Mushroom extract respectively (group 3). T-test showed statistical significant difference between the two groups (The significance level was set at P ≤ 0.05).

DISCUSSION

DMBA is a polycyclic aromatic hydrocarbon used to induce breast cancer and thus was chosen to induce cancer in the present study. This potent carcinogen induces DNA damage. In the cell, the reactive metabolite DMBA-3,4-dihydrodiol-1,2-epoxide (DMBA-DE) adds adenine and guanine residues to DNA. The conversion of genotoxic metabolites as DMBA-DE is promoted by the action of cytochrome P450 family. CYP1A1 and CYP1B1 are identified as the enzymes that metabolize DMBA to produce DMBA-DE.[42]

DMBA-induced hamster buccal pouch carcinogenesis, is an accepted model for studying precancerous and cancerouslesions of human oral squamous cell carcinoma, since it represents closelymorphological and histological similarity to human neoplasms, as well as, its ability to expresses many biochemical and molecularmarkers that are expressed in human tissues[43]. However, in the present work, it was systemically administered to female rats to mimic the induced breast cancer and oral squamous cell carcinoma model. Immunohistochemical analysis of biomarkers could help to establish a direct association between the morphology and the biomarkers, which can aid in determining their functional relevance.[44]

Oxidation is essential for many living organisms to produce energy to accomplish biological processes. However, oxidative stress resulting from increased release of free radicals and decreased antioxidant status in the target cells and tissues play an important role in carcinogenesis[45]. All organisms possess antioxidant defense and repair systems that are meant to protect them against oxidative damage; yet, these system are insufficient to prevent the damage entirely[46]. Natural products with antioxidant activity may be used to help the human body to reduce oxidative damage. Several types of fruit, vegetables, herbs, cereals, seeds and edible types of mushrooms have been investigated

for their antioxidant and anticancer activities,[47] and this was the main reason why it was chosen in the present work to represent a promising anticarcinogenic agent.

Recently, mushroom consumption has been markedly increased throughout the world and involves a variety of edible species like Volvariellavolvacea, Agaricusbisporus and Calocybeindica which are abundant in India[48, 49]. For thousands of years, medicinal properties of mushrooms have been well known in Eastern Asia[50]. The involvement of several mushrooms in breast cancer prevention is of special interest including the popular mushrooms Ganodermalucidum that has been widely used for the general improvement of health in Asia. Chemical investigations on the fruiting bodies and spores of G. lucidum reveal that they contain various bioactive substances[51]. These bioactive compounds belong to the group of polysaccharides and antioxidants, which protect the body against free radicals that destroy body cells inducing disease and eventually cancer.[52]

In this study, our aim was to histologically evaluate the effect of Mushroom extract on (DMBA)-induced lip carcinogenesis in albino rats, in an attempt to study its effect in a model close to human squamous cell carcinoma. This effect was analyzed by co-administrating the Mushroom extract powder following the systemic administration of DMBA only for three weeks to emphasis its carcinogenicity. Histochemical analysis of specimens obtained from sacrificed animals treated with DMBA alone emphasized the signs of carcinogenicity in the form of dysplasia in the epithelium of the labial mucosa and skin sidewith obvious invasion of the underlying connective tissue with malignant epithelial cells. The malignant epithelial cells showed hyperchromatism and pleomorphism. The connective tissue showed masses of malignant epithelial cells, inflammatory cells and dilated blood vessels. These findings were in agreement with **Arroyo-Acevedo et al.**[53] who

found an altered cellular architecture and enlarged hyperchromatic epithelial cells following DMBA administration.

In the present work, we observed an apparent increase in the thickness of thekeratin layer of Group 2 especially on the skin side. This was coincident with **Akira Ohkoshi et al.**[54] who investigated the keratinization in tongue and upper aerodigestive tract carcinogenesis. They noticed an increase in both epithelial and keratin thickness, and referred that to the action of transcription factor Nrf 2, which is activated in response to environmental insults and oxidative stress conditions. This factor has a pivotal role in cellular defense against toxic electrophiles and oxidative stresses in the form of thickened keratin layers as a mechanical defense mechanism and a chemo-preventive feature.

Regarding group 2 our results showed dilatation of blood vessels in the connective tissue and massive inflammatory cell infiltration in both lamina propria of submucosa and dermis of the skin accompanied by loss of connective tissue in some areas. This came in accordance with **Coussens and Werb**[55] who noticed severe skininflammation generated upon DMBA administration and attributed that to the correlation between chronic inflammation and cancer.

On the other hand, histopathologic analysis of specimens obtained from animals treated with DMBA for three weeks followed by DMBA & Mushroom extract for another 3 weeks showed a reduction in the dysplastic features of the epithelium of the labial mucosa and skin side of the lip. There were no signs of epithelial invasion of the connective tissue and this was represented by an intact basement membrane with less signs of inflammation and congestion of blood vessels in the connective tissue. This could be referred to what has been postulated by **Krishnamoorthy and Sankaran**[56] that oyster mushroom extract was a potential anticancer agent in different cancer

cell lines and experimental animals. Furthermore, **GuYH.** [57] screened the anticancer activity of some mushroom species against human androgen-independent prostate cancer PC-3 cells and they found that a water-soluble extractproduced the most significant cytoxicity and induced apoptosis in PC-3 cells in a dose dependent manner.[57]

Another study on an aqueous polysaccharide extract from the edible mushroom *P.ostreatus* concluded that this type induces anti-proliferative and pro-apoptotic effects on HT-29 colon cancer cells, owing to the presence of newly identified lowmolecular weight α-glucan with promising anti-tumorigeneticproperties, and demonstrated its direct effect on colon cancer cellproliferation via induction of programmed cell death[58].Moreover, the hot water extract of *P. ostreatus*also showedsuppression in proliferation of MCF-7 human breast cancer cells.[59]

In our study, the skin side of the lip of group 3 still showed some signs of epidermal disruption in the form of intracellular edema, also very few epithelial cells invaded the dermis close to the basement membrane. These findings might correlate to the need for a longer duration or a higher dose of the treatment used.

Furthermore, studying the differences among the three groups concerning the immunoexpression of COX-2, the highest expression could be detected in group 2 in which the sacrificed animals were treated with DMBA alone. Cyclooxygenase-2 (COX-2) has been proposed to play an important role in the promotion of carcinogenesis, tumor invasion and angiogenesis. The increase in COX-2 expression was previously proved in several studies to correlate with higher grade of malignancy and increased rate of mortality in different cancer types. Studies also proved the cells with increased migration ability had higher expression of COX-2 with increased motility and invasiveness.[60, 61]

The effects of COX-2 on human oral cancer

cells are largely unknown. In 1991, **Thun et al.**[62] undertook an epidemiological study, which reported that regular aspirin intake at low doses reduces the risk of colorectal cancer and suggested the link between COX enzymes and cancer development. A large body of evidence now indicates that elevated levels of COX-2 are present in the majority of colorectal carcinomas[63, 67] and in a subset of adenomas[63, 64]. The observation that COX-2 expression is up-regulated in both pre-malignant as well as malignant colorectal tissue is regarded as particularly significant, having potential implications for both the prevention and treatment of cancer [68].

Tumor invasion and metastasis are the main properties determining the aggressiveness of human cancer. Mortality in patients with cancer depends mainly on the metastatic spread of malignant cells to distant organs [69]. To facilitate cell motility and allow metastatic spread, malignant cells need to change their cell to cell adhesion properties, reorganize the extracellular matrix environment and rearrange the cytoskeletons of these cells [70]. The interaction of COX-2 with its specific EP receptors on the surface of cancer cells was found to induce cancer invasion [71]. A previous study showed that COX-2/PGE2 promotes cell migration and the expression of ICAM-1 in human oral cancer cells.[61]

NSAIDs such as aspirin have been used traditionally as analgesics and anti-inflammatories for centuries and are some of the world's most widely used drugs.In the early 1970s, the enzyme prostaglandin G/H synthase, now more often referred to as COX, was elucidated as an inhibition target for aspirin and other NSAIDs [72]. Since this discovery, there has been a great deal of interest in identifying the components of the COX pathway as well as its functions in both physiological and pathological conditions. [73]

COX enzymes play key roles in the biosynthesis

of prostaglandins from arachidonic acid following its release from the plasma membrane by the action of phospholipase-A2[73].Prostaglandins are important for a large number of normal physiological processes in a broad range of tissues. These include the modulation of immune responses, protection of the gastrointestinal mucosa, maintenance of renal homeostasis and the regulation of blood clotting. Furthermore, prostaglandins also function in pathological conditions where they can promote inflammation, swelling, pain and fever.[72, 73]

Reduced expression of COX-2 in cases treated with DMBA followed by mushroom extract (group 3 in this study) is a sign of better prognosis and less probability for the dysplastic cells to invade the surrounding tissues. On comparing the mean and standard deviation of COX-2 expression in group 1 (-vecontrol group) and group 3 (DMBA + Mushroom extract), no significant difference was found. This finding proves that the addition of mushroom extract reduces the dysplastic changes of the epithelial cells and may play a role in decreasing their ability to invade the surrounding tissues.

To conclude, our study presents a possible natural source (Mushroom extract) that may limit the carcinogenic effect of a known carcinogenic agent through suppression of the inflammatory reaction and the invasive power. Further studies are recommended to clearly establish the clinical evidence of mushroom anticancer activities.

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