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EFFECT OF MUSHROOM EXTRACT ON 7,12-DIMETHYLBENZ[A]ANTHRACENE INDUCED SALIVARY GLAND PATHOSIS IN ALBINO RATS

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ABSTRACT

Salivary glands are essential structures for the wellbeing of an individual. Salivary gland tumors represent a diagnostic challenge. Following the treatment protocol for malignant salivary gland tumors, the majority of patients experience decreased salivation. Regeneration after the damage resulting from the treatment protocol, is believed to be through the transient activation of the Wnt/β-catenin pathway which preserve the stem/progenitor pool and allow for regeneration. Medicinal mushrooms have been tried in medicine for centuries. They have been reported to have anti-inflammatory, cardio-protective, hepatoprotective, and anticancer properties. **Aim of the study:** to evaluate the effect of mushrooms on the salivary glands of albino rats following DMBA induced pathological changes. **Materials and methods:** The study comprised three groups each of 12 albino rats. Group I was the control group, group II was given DMBA and group III was treated with mushrooms, following DMBA adminsteration. All groups were assessed histopathologically (H&E) and immunohistopathologically (PCNA). **Results:** Group II exhibited variable histopathological signs from apoptosis to inflammation, allergy and dysplasia. Group III showed absence of some of the previous signs and a significantly higher PCNA expression. **Conclusion:** mushrooms may help in the regeneration of acinar cells through the activation of the progenitor cells. It also may have a cancer- protective role.

KEY WORDS: Salivary glands, DMBA, mushroom, Progenitor cells, reactive oxygen species

INTRODUCTION

Salivary glands are essential structures for the wellbeing of an individual. Those who lose their salivary gland function due any pathological condition, are probably the most aware of their importance. Saliva has several roles from simple lubrication action to an immunological role. The acini are the salivary secretory units. There are three major salivary glands; the parotid, submandibular and sublingual glands. Mucous acini are made up of elongated cells with foamy cytoplasm and a peripherally located nucleus. The nucleus is compressed at the basal end of the cell by the large

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amounts of mucous within the cytoplasm. Serous acinar cells are more angular/ triangular in shape with round nuclei. The serous acini contain abundant amylase secretory granules that are Periodic acid–Schiff (PAS) positive[1, 2].

Both types of acinar cells have myoepithelial cells located between the secretory cells and the basement membrane, they are also present related to the intercalated ducts. The functions of myoepithelial cells are mainly contractile as they provide support, maintain the secretory units integrity, and push saliva through the ductal system avoiding stagnation. Some research also suggest that myoepithelial cells play a role in tumor suppression, through the secretion of tissue inhibitor of metalloproteinase-1 (TIMP), maspin (inhibitor of tumor growth and invasion) and α1-antitrypsin[1,3]. Myoepithelial cells are believed to be multipotent and to take part in acinar cells differentiation during embryonic development. They have been reported by many authors to take part in acinar regeneration after atrophy, as well[4-6]. It is assumed that acinar cells do not proliferate, and that their renewal depends on progenitor stem cells, even though some authors state otherwise [6-8].

Salivary gland tumors represent a diagnostic challenge. This is attributed to the numerous salivary gland tumor entities, the occasional morphologic overlap between them, and the rarity of a lot of the defined tumor types[9-11]. Surgery is generally the mainstay of malignant salivary gland tumors management, as it serves a diagnostic as well as a therapeutic purpose. Postoperative radiotherapy is useful for tumors at risk of locoregional recurrence. The role of chemotherapy is as yet not well established and investigations of targeted therapy are still at an early stage[12].

Following the treatment protocol for malignant salivary gland tumors, the majority of patients experience decreased salivation, which greatly affects their quality of life. The serous acini are documented to be more prone to the detrimental effects associated with cancer treatment, than are the mucous acini[13, 14]. The greater effect seen in serous cells is thought to be due to the generation of free radicals from the transition of minerals (mainly copper and iron) in their secretory granules, these are thought to be located close to the nucleus and may result in DNA damage [15-18]. Regeneration after injury resulting from treatment protocol, is suggested to be through the transient activation of the Wnt/β-catenin pathway which preserve the stem/progenitor pool and allow for regeneration[9, 19, 20].

Now-a-days several researches in cancer therapy are tackling the application of repurposed "old" drugs and phytochemicals, both of which aim to reduce the use and side effects of the conventionally used cytotoxic drugs. Mushrooms have been tested in medicine for centuries. They have been reported to have anti-inflammatory, cardio-protective, hepatoprotective and anticancer properties. Their application in oncology is popular, as several researches documented their use in cancer prevention and therapy and as an adjuvant to traditional cancer therapy[21, 22]. Shiitake, Maitake, Reishi and Turkey Tail Mycelia are all types of mushrooms that have given promising results in cancer prevention/ therapy[21, 23].

Mushrooms are classified under the kingdom "Fungi", which reproduce as spores. Mushrooms have many bioactive components which comprise polysaccharides, fats, glycosides, proteins, volatile oils, tocopherols, alkaloids, phenolics, folates, flavonoids, ascorbic acid enzymes, organic acids, carotenoids, polysaccharides, terpenoids, and nucleotides. Most of these compounds are considered to be natural anti-oxidants, which are in very high demand these days. Medicinally, the most frequently acknowledged ingredient in mushrooms, is the Beta-glucan (B-glucan)[22, 24, 25].

B-glucans are powerful immune-modulators of both the innate and the adaptive immunity. They modulate the release of several cytokines such as; interleukin (IL)-12, IL-6, tumor necrosis factor

(TNF)-α, and IL-10. Moreover, the polysaccharides in mushrooms are potent antioxidants and were found to be able to down regulate the PI3/AKT signaling pathway[26]. Also, B- glucan from mushroom were reported to have a modulatory effect on the Wnt/β catenin signaling, which was found to enhance the power of regeneration by the skin[24].

Proliferating cell nuclear antigen (PCNA) is detected in all the phases of the cell cycle and is especially prominent during the S phase. Aberrations in PCNA expression is a documented feature of tumor cells. Upregulation of PCNA, is perceived as evidence of uncontrolled proliferation[27].

The present work aims to evaluate the potential therapeutic effect of a combination of five medicinal mushrooms on 7,12-Dimethylbenz[a]anthracene (DMBA) induced salivary gland pathology of albino rats.

METHODOLOGY

Animals and Materials

The entire 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 about 180-200 gm were used in this study.

DMBA was purchased from Sigma Aldrich chemical company. It was supplied in powder form, which was dissolved in sesame oil to form an emulsion. Cancer was induced by a single weekly intragastric administration of DMBA (65 mg/kg of body weight) in 1.0 ml of sesame oil(28, 29).

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 form and administered by a dose equivalent to 200mg/kg body weight and dissolved in distilled water (an inert solvent)[30].

Experimental design

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

Group 1: Control (–ve control group in which each rat was given 1 ml sesame oil viagastricgavage 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+Mushroom (Experimental group in which each rat was given DMBA emulsion for 3 weeks then DMBA co-administered with the mushroom extract (dissolved in distilled water) for the another 3 weeks via gastric gavage.

The animals were sacrificed by cervical dislocation. For histopathologic examination, the salivary glands were dissected free 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. 4-5μm thick sections 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).

H&E staining

The sections were then stained with H&E solutions (Sigma, St. Louis, MO, USA)to verify histopathological findings.

Immunohistochemical staining

Paraffin embedded tissue sections from the salivary glands of the rats included in the study, were dewaxed and rehydrated through graded ethanol. Endogenous peroxidase was blocked by incubation with 3% H2O2 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 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 (PCNA- 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. Photomicrographs were captured at a magnifications of x20 and x40. All steps for immunohistochemical quantitative evaluation were carried out on photomicrographs captured at a magnification of x20 using image analysis software (Image J, 1.41a, NIH, USA).

Statistical analysis

The area fraction of immunopositivity with PCNA antibody was calculated. The data were 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. Independent t test was used for comparison between groups. The significance level was set at $P \leq 0.05$.

RESULTS

H&E

Group I

Mucous acini from the control group were tubule shaped, the secretory acinar cells were flattened with foamy cytoplasm. They featured flat nuclei which was compressed toward the basal cell surface by the mucous in the apical cytoplasm (fig. 1). On the other hand, serous acini of the same group, were more spherical with a narrow lumen, serous acinar cells were pyramidal in shape with round nuclei in the middle of the cytoplasm. The apical cytoplasm of the serous acinar cells was granular (fig. 2).

The intercalated duct was made up of simple cuboidal epithelium that was in part covered by myoepithelial cells. Intercalated ductal cells had central nuclei with small secretory granules in the apical cytoplasm. The ductal cells of the striated ducts were columnar with central nuclei, their basolateral membrane had multiple foldings, giving it the characteristic "striated" impression. (figs. 1&2)

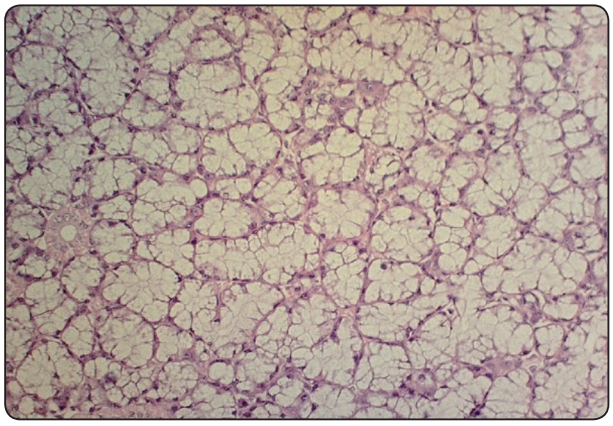

Fig. (1) Phtomicrograph showing mucous acini from the control group. Note the flattened apically situated nuclei. (H&E, original mag.x 20)

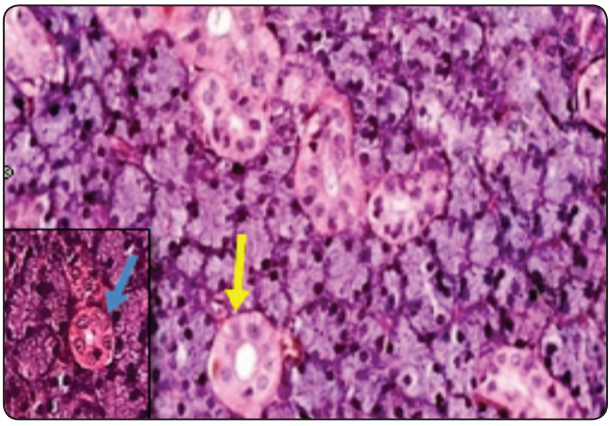

Fig. (2) Phtomicrograph showing serous acini (Blue Arrow) from the control group, and striated ducts (yellow arrows). (H&E, original mag.x 20)

Group II:

The group given DMBA exhibited diverse responses, where some rats exhibited signs consistent with inflammation, degeneration and/ or allergic reactions. While, other rats showed some histological features of dysplasia that was especially prominent in the serous acini these serous acinar cells showed nuclear pleomorphism, hyperchromatism, increased nuclear cytoplasmic ratio with concomitant distortion of acinar outlines but with preservation of lobular architecture (fig. 3). Alternately, others exhibited cytoplasmic vacuolation and eosinophilic condensation of the cytoplasm. In the latter specimens, the acinar srchiture appeared to be lost (fig. 4).

At the same time, the mucous acinar cells showed milder changes, where their nuclei were open faced and vesicular with prominent nucleoli, rather than being flat and compressed (fig. 5).

The ductal system of this group was also affected as there were signs consistent with sialadenitis, inflamation and mast cell infilteration in some specimens (figs. 6-9), while others showed signs of degeneration and apoptosis. Apoptotic cells exhibited morphological features like cell shrinkage, condensed nuclei and nuclear fragmentation(fig.10)

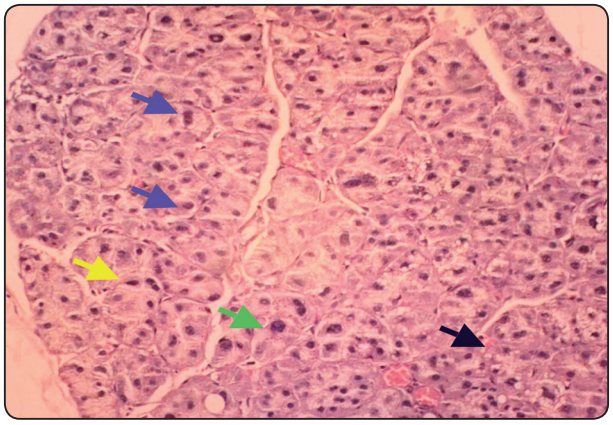

Fig. (3) Phtomicrograph showing serous acini from the DMBA treated group. The cells exhited nuclear pleomorphism (blue arrow), hyperchromatism (yellow arrow), increased nuclear cytoplasmic ratio (green arrow) with concomitant distortion of acinar outlines but with preservation of lobular architecture. Congested blood vessels were also noted (black arrow).(H&E, original mag.x 40)

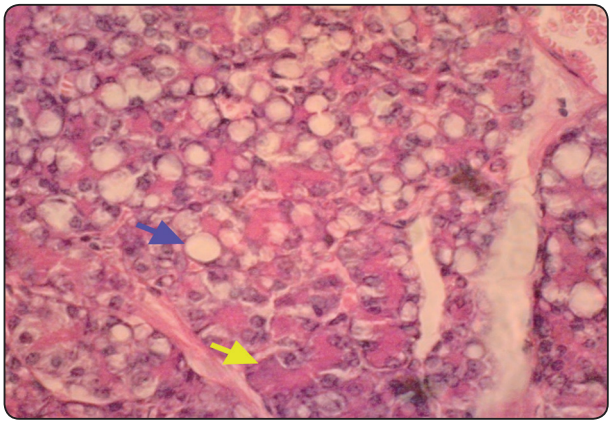

Fig. (4) Phtomicrograph showing serous acini from the DMBA treated group. This specimens exhibited destruction of the acinar architecture some cytoplasmic vacuolations (blue arrow) and/ or hyalanization marked eosinophilic condensation of the cytoplasm (yellow arrow) (H&E, original mag.x 40)

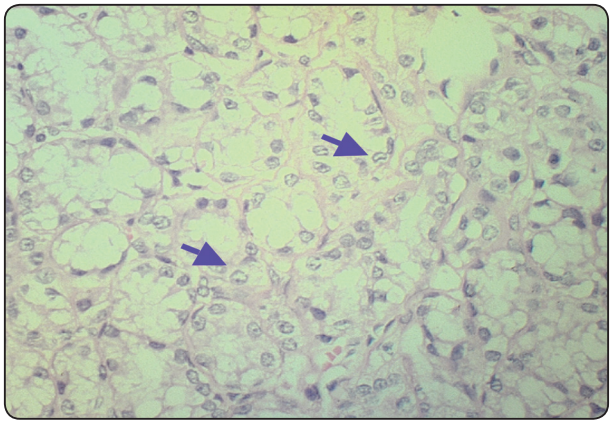

Fig. (5) Phtomicrograph showing mucous acini from the DMBA treated group. Note the majority of the nuclei of the acinar cells are open faced, vesicular with prominent nucleoli.(blue arrow) (H&E, original mag.x 40)

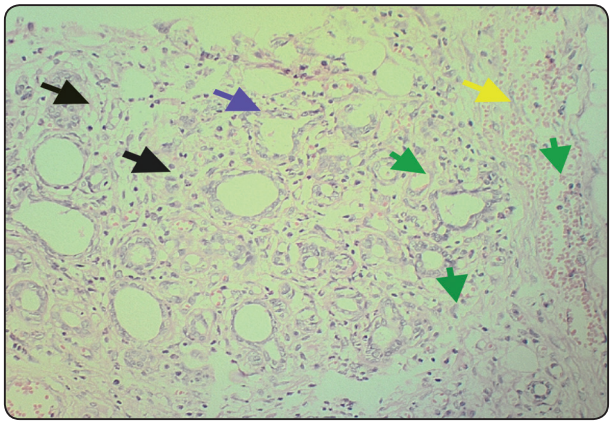

Fig. (6) Phtomicrograph showing interlobular ducts from the salivary glands of the DMBA treated group. This specimens exhibited distortion and dilatation of the ducts (blue arrow). Vasodilatation and congestion of the blood vessels (yellow arrow). Also, few PMNL`s were noted in the blood vessels and within the stroma of the studied pecimen( green arrow), lymphocytes and plasma cells (black arrow) were also seen infliterating the stroma (H&E, original mag.x 20) PMNL`s; polymorphnucleuar leukocytes.

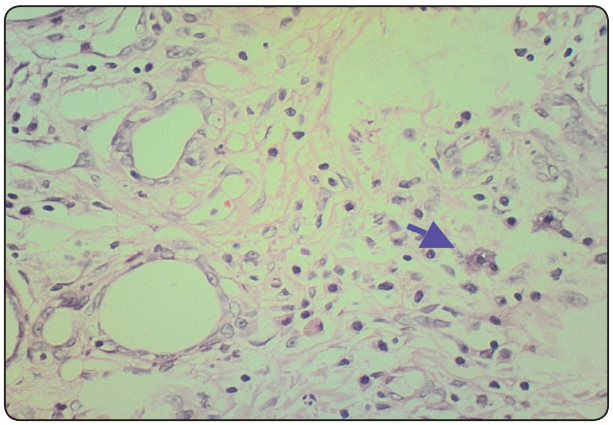

Fig. (7) Phtomicrograph showing macrophages in the salivary glands of the DMBA treated group. (blue arrow). (H&E, original mag.x 40)

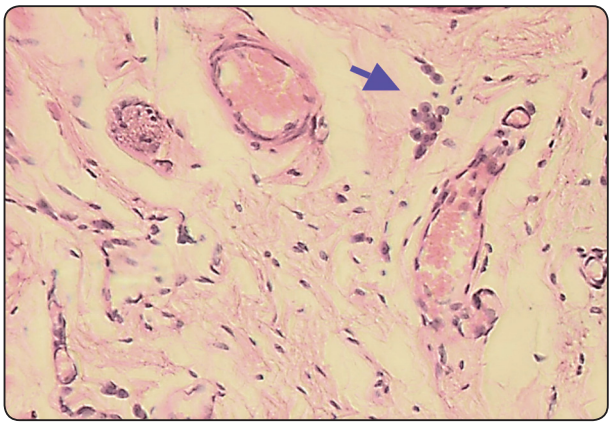

Fig. (8) Phtomicrographshowingaggregate of mast cells proximal to the blood vessels of the salivary glands of the DMBA treated group (blue arrow). (H&E, original mag.x 40)

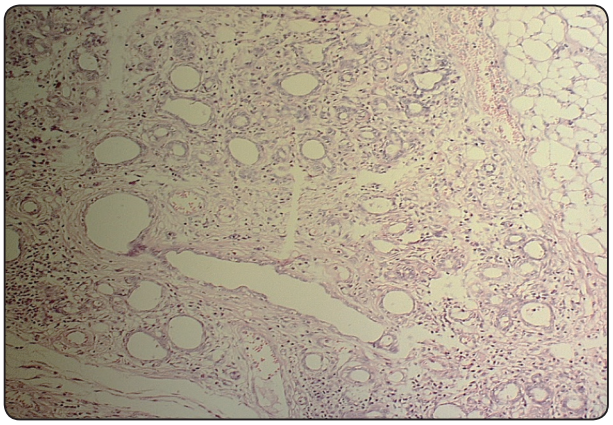

Fig. (9) Phtomicrograph showing features consistent with sialadenitis in the salivary glands of the DMBA treated group. Note the ductal dilatation and inflamatory cell infilterate (H&E, orig. mag.x20).

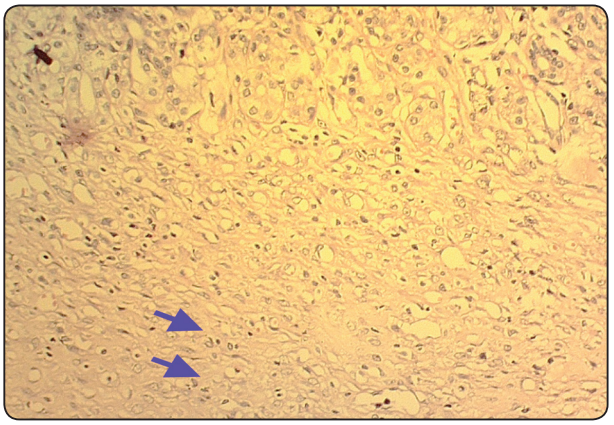

Fig. (10) Phtomicrograph showing salivary glands of the DMBA treated group. Note the fibrosis and degeneration with some cells demonterating signs of apoptosis(blue arrow). (H&E, orig. mag.x20)

Group III

The mucous and the serous acini of this group were almost normal. The mucous acinar cells showed flattened nuclei and the acinar outlines were normal. Some congestion in the blood vessels was noted in between the acini (fig. 11). On the other hand, in the serous acini some focal area reatined some nuclear pleomorphism in the acinar cells (fig. 12).

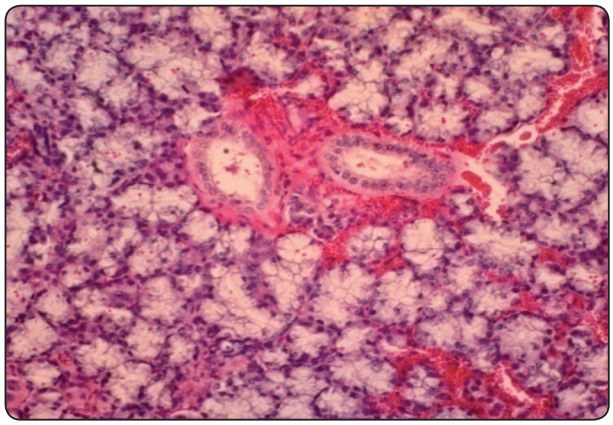

Fig. (11) Photomicrograph of group III rats showing mucous acini with normal histological findings, except for the presence of some congestion of the blood vessels. (H&E, orig. magx20)

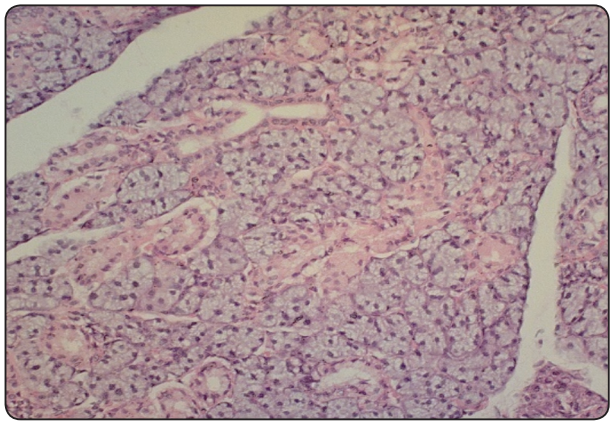

Fig. (12) Photomicrograph of group III rats showing serous acini with mild nuclear pleomorphism and hyperchromatism. (H&E, orig. magx20)

PCNA immunohistochemistry

Assessment of area fraction of immunopositivity against PCNA monoclonal antibody

Statistical evaluation of the area fraction of immunopositivity was found to be highest in group III (mushroom) followed group II (DMBA) then group I (control)(fig. 13). Statistically significant differences were detected by ANOVA and was found to be between group III and both of the other (groups Iand II). No statistically significant difference was noted between groups I and II. (table 1)

TABLE (1) Showing t- test and ANOVA results for the studied groups regarding the area fraction of immunopositivity with PCNA

Groups		P value	ANOVA
Control	DMBA	1.000 N.S	26.751 P <0.001
Control	Mushroom	< 0.001 Highly sig.	
DMBA	Mushroom	<0.001 Highly sig.	

p≤0.05

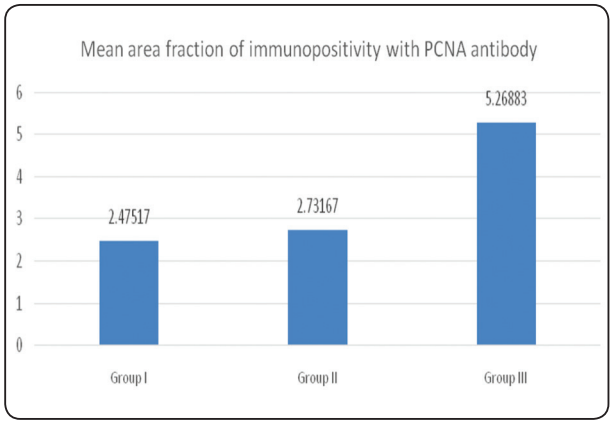

Fig. (13) Column chart showing Mean area fraction of immunopositivity with PCNA antibody in the studied groups

Immunohistochemical evaluation of the studied groups showed that there was no to very little immunopositive reaction in groups I and II. (figs. 14-17). However, some sections in group II showed PCNA immunopostivity in inflammatory cells infiltrating the parenchymal cells of the gland and not the parenchymal structures themselves. These immunopositive reaction appeared to be cytoplasmic and nuclear. (figs. 18&19).

Group III exhibited immunonegative serous and mucous acinar cells. The ductal cells demonstrated some immunopositivity. Granular cytoplasmic immunopositivity was noted, as well, in the cells peripheral to both types of acinar cells. These immunopositive cells appeared to be spindle to stellate in morphology. (figs. 20-23)

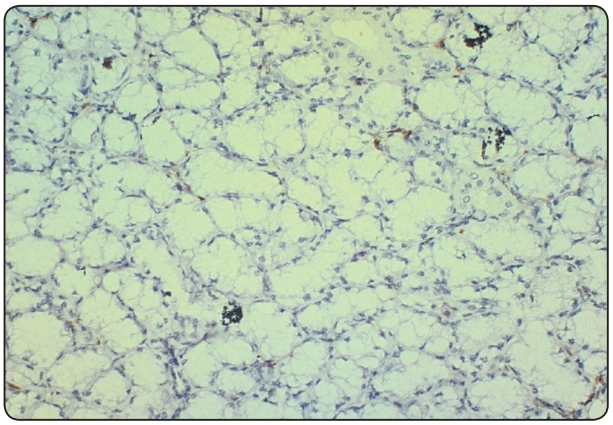

Fig. (14) Photomicrograph showing immunonegative mucous acini from group I (PCNA, orig. magx20)

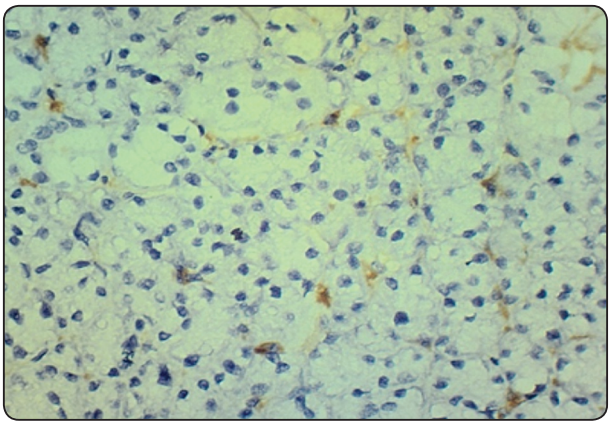

Fig. (15) Photomicrograph showing immunonegative serous acini from group I. Almost the acinar cells were immunonegative (PCNA, orig. magx 40)

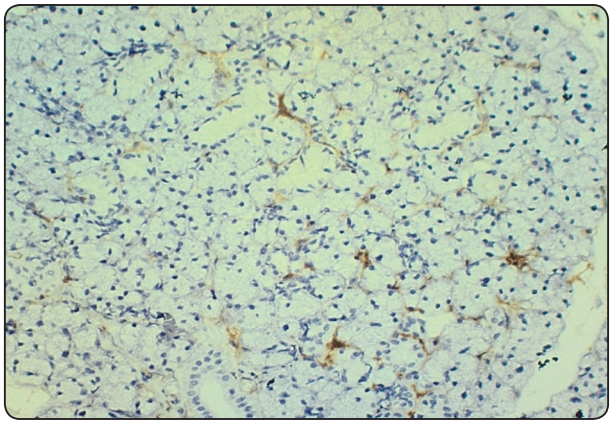

Fig. (16) Photomicrograph showing mucous acini from group II, the cells were immunonegative. (PCNA, orig. magx20)

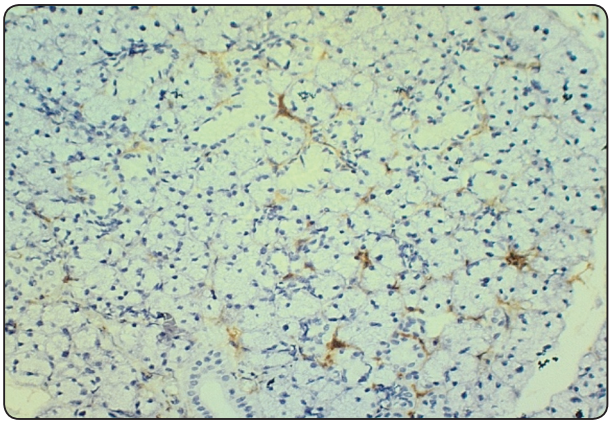

Fig. (17) Photomicrograph showing immunonegative serous acini from group II (PCNA, orig. magx20)

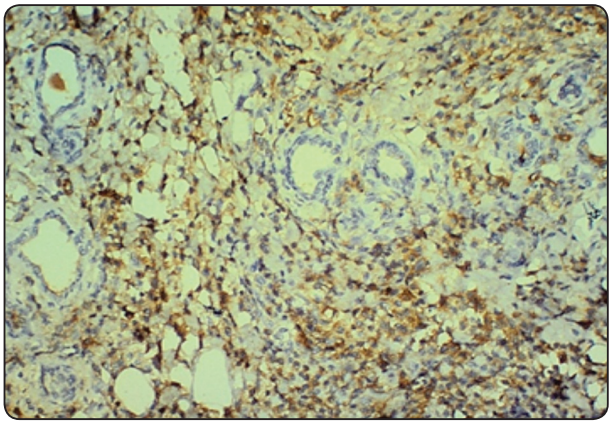

Fig. (18) Photomicrograph showing immunonegative ductal cells and a dense infiltrate of immunopositive cells in the stroma (green arrow) from group II (PCNA, orig. magx20)

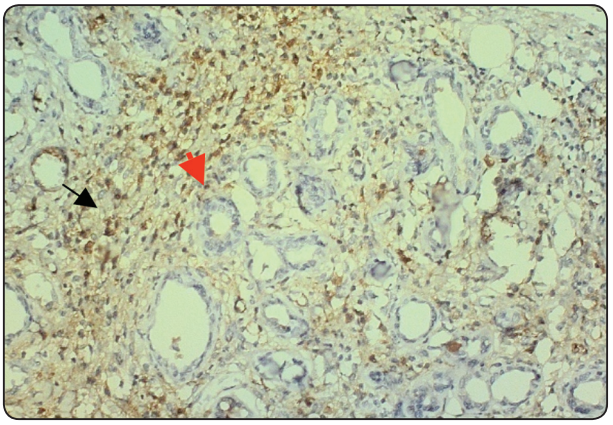

Fig. (19) Photomicrograph showing immunonegative ductal cells (red arrow) with a dense immunopositive infiltrate of inflammatory and connective tissue cells (black arrow) from group II (PCNA, orig. magx20)

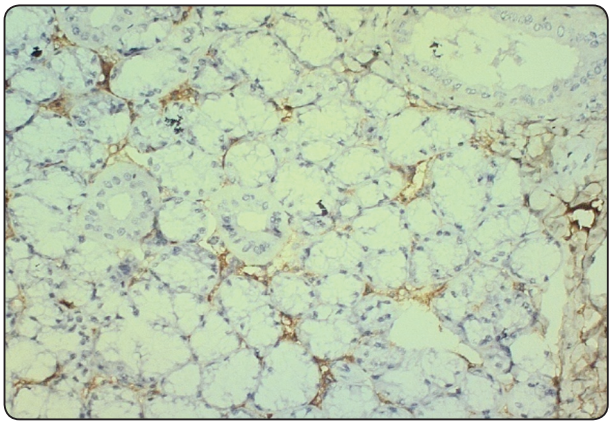

Fig. (20) Photomicrograph showing immunonegative mucous acinar cells with focal immunopositivity between the acini in group III (PCNA, orig. magx20)

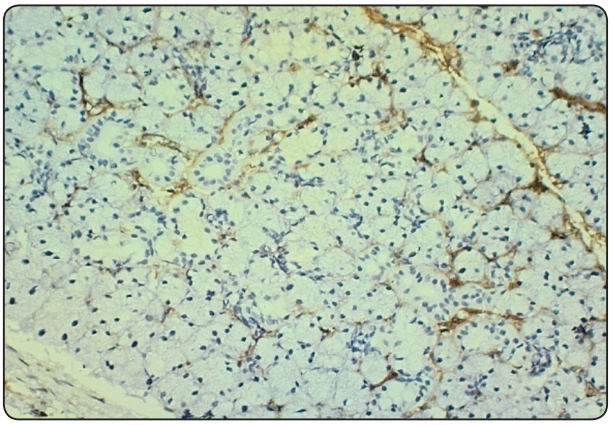

Fig. (21) Photomicrograph showing immunonegative serous acinar cells with focal area of immunopositivity. in group III (PCNA, orig. magx20)

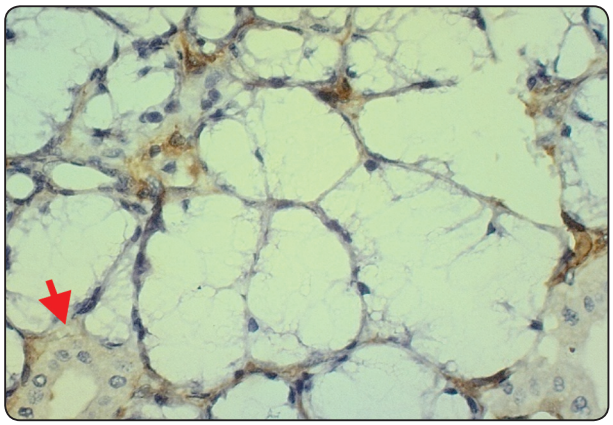

Fig. (22) Photomicrograph of salivary glands of group III showing immunonegative mucous acinar cells with focal immunopositivity in the cells peripheral to the acini and the ductal cells (red arrow). (PCNA, orig. magx40)

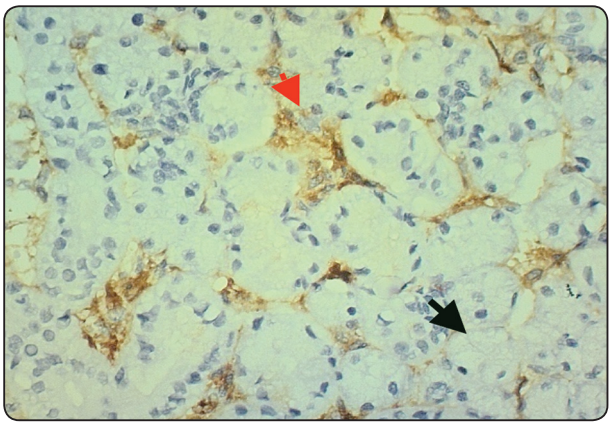

Fig. (23) Photomicrograph of salivary glands of group III showing, immunonegative serous acinar cells. Granular cytoplasmic immunopositivity was noted in the cells peripheral to the acini. These immunopositive cells appeared to be spindle (red arrow) to stellate (black arrow), in morphology. (PCNA, orig. magx40)

DISCUSSION

This study was performed to evaluate the effect of medicinal mushrooms on the salivary glands of DMBA treated albino rats. Salivary gland tumors are diverse and most of the entities are exceedingly rare. Moreover, there are constant discoveries regarding the classification, pathogenesis, differential diagnosis as well as management of the salivary gland tumors[11], like the recently described mammary analogue secretory carcinoma of the salivary glands[31]. The chemical carcinogenesis model was introduced where tumors are `deliberately` induced using chemicals in lab animals, which gives more room for research.

Rats were the animals of choice for the current work in accordance to the work done by ShklarG. and Sonis S.T.,1975[32], MainentiP., et al, 2008[19] and Cao X, 2000[16], who used rats for the induction of salivary gland carcinogenesis. The carcinogen used in these models was DMBA, a polycyclic aromatic hydrocarbon (PAH). DMBA is a common laboratory carcinogen. It is cheap and easily available, making it an excellent candidate for induction of carcinogenesis[17].

DMBA is an indirectly acting carcinogens, it's activation is mediated by cytochrome P450 enzymes. Bioactivated metabolites generate DNA adducts; through targeting several genomic sites, with guanine and adenine bases. Moreover, DMBA was found to increase proapoptotic signals and signs of oxidative stress, due to reactive oxygen species (ROS) liberation. ROS readily reacts with other molecules like lipids, proteins and DNA causing their damage[16, 19].

In this study, DMBA was introduced via gastric gavage in accordance to Verma, A.K., et al, 1988, Benakanakere, I, et al, 2006, Yang, Y., et al, 2005 and Yang, S. H., et al, 2013, who used the same method for the induction of mammary gland carcinogenesis. In the current work this protocol was adopted in the belief that salivary glands may respond in a similar manner to the mammary glands. Other researchers also reported that, this method was effective in inducing cancers in different tissues, like the oral mucosa[28, 33-35]. The durations followed in this study were in accordance to Papaconstantinou, A.D, et al, 2006, who administerd DMBA for six weeks and stated that gastric gavage of DMBA "induces nearly a 100% incidence of carcinomas within a relatively short latency period"[36].

Mushroom extract mixture of (Shiitake, Maitake, Reishi and Turkey Tail Mycelia), effect on salivary gland pathological changes induced by DMBA is evaluated in this study. Medicinal mushrooms are gradually becoming recognized for their ability to treat a wide variety of diseases.They have the added benefit that they can be used straight away, without having to re-pass through phases-I/II/III clinical trials like other experimental medicine[25].

H&E was used in the present work to evaluate the histomorphological features of the studied specimens. Furthermore, PCNA was evaluated immunohistochemically to assess the presence of abnormal mitosis in the parenchymal cells in the group given DMBA and the possible change in pattern of expression after mushroom treatment.

The results of this study showed that after the ingestion of DMBA through-out the experimental period, the rats exhibited different reactions that were observed histologically. Some rats exhibited an inflammatory reaction with infiltration of inflammatory cells ranging from PMNs and macrophages to lymphocytes and plasma cells, fibrosis and dilatation of the salivary gland ducts. This may be attributed to the release of ROS which activate NF-κB signaling, which in turn results in induction of an inflammatory response[37]. NF-ϰB is reported to take part in both the innate and adaptive immune responses[20].

Others developed a histological picture consistent with an allergic reaction, where there were dilated and congested blood vessels and focal presence of mast cells. These findings were similar to those of Crawford G, et al., 2018, who stated that, DMBA administration was associated with the establishment of an IgE response that proved

to actually protect from carcinogenesis[38]. The contribution of mast cells to carcinogenesis appears to vary from pro-carcinogenic to anti-carcinogenic, probably owing to the differences in carcinogenesis models used in different studies[39].

Moreover, some of the studied biopsy specimens showed signs of degeneration and apoptosis. This may be explained by the fact that DMBA binds to the DNA and that in some rats the cell cycle check points retained their function and induced apoptotic signaling. Also, this may be attributed to the effect of ROS which damage DNA, proteins and lipids and hence push the cells through apoptosis[18].

The serous acini of the majority of the specimens exhibited signs that were consistent with histopathological dysplasia, without the development of any malignant salivary gland tumor type. On the other hand, the mucous acini seemed to be even more resistant to the effects of DMBA, where they only showed change in the nuclei as they changed from being flattened to become round, open faced and with a prominent nucleoli. These results were consistent with those of Maciejczyk M, et al, 2018 and Redman RS, et al, 2008, who stated that serous acini were more harshly affected by oxidative stress than the mucous acini[15, 40]. This may be explained by the fact that the minerals present in the serous secretory proteins produce free radicals which may result in DNA damage[15]. Also, this may possibly be due to the fact that, DMBA has the ability to activate cytokine-driven TNFα production[41].

The open faced nuclei as those noted in the mucous acini in group II of this study, is generally a sign that the cells are transcriptionally active with large areas of euchromatin[13]. This may a reaction to the ROS production or it may be due to the attempt of the mucous cells to increase secretion to as to compensate for the malfunctioning of the serous acini, depicted histopathologically in this study[40].

In group III, after mushroom treatment the mucous acini returned to their normal histomorphological features, the signs of inflammation and dysplasia were greatly reduced. This may be due to the fact that mushrooms induce DNA repair, are anti-oxidants and immune-modulators via down regulating IL6 and TNFα [21-23].

The theory that acinar cells of the salivary glands are stable cells and that their replacement is via differentiation of progenitor cells, is still under debate. In the present study however, it was noted that the control group (group I) the acinar cells were almost entirely immunonegative with PCNA antibody, indicating the absence of proliferation. Immunohistochemical evaluation of PCNA expression showed that group I and II had significantly lower PCNA expression than group I. This may be due to the fact that mushrooms are known to inhibit the ROS production allowing for regeneration, after the initial stress induced by DMBA administration. The expression was noted not to be in the acinar cells but was in the myoepithelial cells surrounding both the acinar cell types. This may be an indicator that mushrooms not only have an anti-oxidant effect on the salivary parenchyma but they possibly also allow for regeneration and replacement of the damaged acinar cell from progenitor cells. Other reports documented similar findings, as they stated that myoepithelial cells aid the atrophied salivary glands in recovering their original shapes with acinar cell regeneration as myoepithelial cells are able to mitotically proliferate, confirmed by upregulation of PCNA expression[4, 5, 14].

Other researchers documented similar results on the effect of mushrooms, which was attributed to the beneficial effects of β-glucan. This effect may be indirect through the activation of several cytokines from macrophages that aid in proliferation, angiogenesis and re-epithelialization, or direct via its` influence on keratinocytes and fibroblasts[42]. It should be noted as well that, in the present work, the slightly higher PCNA expression noted in group II over group I may be attributed to the expression of PCNA in the inflammatory and stromal cells rather than the parenchymal cells itself.

CONCLUSIONS

This protocol using DMBA failed to produce a frank malignant tumor in the salivary gland tissue, but produced signs consistent with cellular dysplasia. The serous acini are more liable to the detrimental effects of DMBA. Mushrooms probably have a regenerative effect and may aid in healing of injured tissues. Mushrooms apparently also have a cancer protective effect which is thought to be due to its` antioxidant constituents

RECOMMENDATIONS

Adjustments to the carcinogenesis model used in this study is required

Mushroom extract is a remarkable agent that needs further pre-clinical and clinical research regarding its` application in tissue regeneration and as a prophylactic/ anti-cancer therapy.

Conflict of interest

None to declare

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