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Aescin serves to protect cell surface architectural abnormalities on 7,12-Dimethylbenz[a] anthracene (DMBA) induced oral carcinogenesis

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Abstract

Oral squamous cell carcinoma is the most common malignant tumor of the oral cavity accounting for 90% worldwide, with India representing nearly a third of the overall burden and having the second-most elevated number of cases. Aescin, a pentacyclic triterpenoid saponin blend derived from Aesculus hippocastanum L (horse chestnut) and is being used as herbal remedies, has anti-edematous, anti-inflammatory, and antitumor pharmacodynamic abilities. In the present investigation, Tumors were triggered by applying 0.5% % DMBA orally to the hamster buccal pouch (HBP) thrice a week for 14 weeks, and Aescin was administered orally in the alternative days. We observed, the status of cell surface abnormalities by evaluating the glycoproteins (Mucins), collagen fiber, and muscle fibers via histochemical staining techniques include Periodic acid-schif (PAS) used to hit upon glycogen-inclusive polysaccharides and mucosubstances, Picrosirius red dye collagen staining techniques for the diagnostic domain to study levels of fibrosis in a whole range of inflammation caused by cancer. Masson's trichrome staining augmented the collagenous fibrous tissue and muscle fibers. Our present results propose that Aescin significantly protects against glycoproteins (Mucins), and collagen breakdown on DMBA induced oral carcinogenesis. We concluded that Aescin significantly protected against cell surface abnormalities in carcinogen-induced buccal pouch experimental oral carcinogenesis.

Keywords: Oral malignancy, aescin, mucin, collagen, histochemical staining**.**

Introduction

Oral cancer is among the most common cancers which are becoming more prevalent throughout the world includes South and Southeast Asia, parts of Europe, regions of Latin America, and the Pacific regions. Oral cancer has become more common in recent years, particularly in developed countries includes Europe and the European Union, United States of America (USA).[1] In 2018, cancers of the lip and oral cavity were reported to have caused 354,864 new cases worldwide, with 177,384 deaths.[2] It has particularly prevalent in South

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Asian countries such as India, Bangladesh, Pakistan, and Sri Lanka, which account for about 40% of newly diagnosed oral cancer cases worldwide, according to the World Health Organization (WHO).[3] The most etiological factor for oral cancer is chewing betel, tobacco, and consuming alcohol.[4] Radiation therapy, surgery, and systemic chemotherapy are widely accessible therapies for oral cancer patients, even though they all have complications that limit their clinical efficacy.[5] Chemo-radiation, on the other hand, provides severe side effects in oral cancer patients. As a consequence, the evaluation and development of generic drugs for oral cancer therapeutics remains controversial.[6]

Aescin, also known as escin, is a saponin mixture stipulated in Aesculus hippocastanum which has anti-inflammatory, and vasoconstrictor capabilities.[7,8] Aescin has also reported pharmacodynamic potential to be utilized for treating chronic venous insufficiency, hemorrhoids, and postoperative edema.[9-11] Most recent research indicates that aescin has anticancer properties in a range of cancers, including lung carcinomas, osteosarcoma, and prostate cancer.[11-13]

Mucins are glycoproteins that function as a selective molecular barrier, and modifications in them are often correlated with cancer, which is generated by cells of the epithelium, or tissue that covers cavities and structures in the body. Furthermore, many cancers of the breast, prostate, lung, pancreas were overexpressed mucins that take advantage of their function in promoting growth and survival.[14,15]

Through the evolution and progression of carcinoma, the collagenous stroma (basic skeleton), undergoes significant changes. To begin with, it can inhibit tumour progression by inhibiting the host immune response or by preventing tumour spread by inducing an abundant collagenous stroma.[16] Furthermore, proteolysis or alterations in collagen composition may assist neoplastic cell mobilization into the stroma, facilitating subsequent invasion and metastasis.[17] In malignancies, though, stromal remodeling develops, which weakens the stroma and allows the tumour to spread quite conveniently.[18]

Histochemical stains are commonly used techniques to aid in tissue analysis. For example, Hematoxylin and eosin (HE) is a technique that creates a distinctive appearance in tissues for a routine inspection. Periodic acid Schiffer (PAS) is one of the most popular histochemical techniques for detecting neutral mucins in tissues.[19] Traditional Masson trichrome stain, which is variations of two or more anionic dyes, is routinely used to detect collagen fibers

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histochemically.[20] Whereas Picrosirius Red (PSR) stain is made up of two anionic dyes, Sirius red F3BA (Direct Red 80) and Sirius red F3BA (Direct Red 80), dissolved in a saturated picric acid solution used to detect collagen selectively.[17]

The study was aimed to look at the histochemical changes in collagen fibers and glycoproteins in different grades of these lesions and evaluate the relationship between these changes, with a focus on histopathological grading.

MATERIALS AND METHODS

Chemicals.

Schiff's Reagent, Sirius red, Picric acid (saturated), Formaldehyde, were be obtained from Hi-media lab Ltd, Mumbai, India, Escin ≥ 95% by TLC (CAS NO. 6805 -41 -0), 7,12-Dimethylbenz[a]anthracen, Hematoxylin and other chemicals of analytical grades were obtained from Sigma Aldrich, USA, and Sisco Chem, Pvt. Ltd. India.

Animals.

A total of 36 male golden Syrian hamsters, aged 8–10 weeks, were obtained from Biogen, Bangalore, India (CPSCEA-certified institute). They were maintained in polypropylene cages under standard conditions and the experiments were carried out according to the guidelines approved by the Institutional Animal Ethics Committee of Animal care (IAEC), Annamalai University (No.160/1999/CPCSEA/10801).

Experimental design

A total of 36 hamsters were randomly distributed into control and experimental groups, among each group containing 6 hamsters (n = 6). In group 1, the animals received only liquid paraffin as a control. For 14 weeks, the animals in groups 2, 3, and 4 were painted with DMBA (0.5 %) in liquid paraffin using a No. 4 sable brush in the right buccal pouch. Animals in group 2 were not provided any other medication. Furthermore, animals painted with DMBA were given Aescin at doses of 20, 40, and 80 mg/kg body weight three times a week on alternate days with the application of DMBA in groups of 3-5. Aescin (40 mg/kg b.wt) alone was given to Hamsters in Group 6. After an overnight fast, the experiment was terminated at the end of the 16th week, and all of the animals were sacrificed.

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Sample collection and preparation

For histochemical staining, collected tissue samples were immediately fixed in 10 % neutral buffered formalin and then embedded in paraffin wax. The paraffin-embedded buccal pouch blocks were processed through routine histological techniques.

PAS special staining (Periodic acidic schiffs)

Place the coverslip on a part of a staining dish, then apply Carnoy's fixative for 10 minutes. Rinse very carefully with several exchanges of deionized water after adding a periodic acid solution to the staining dish for 10 minutes, then wash very carefully with three exchanges of tap or deionized H_2O. Dehydrated in an ascending alcohol solution in the Columbia staining dish (50 percent, 70, percent, 80 percent, 95 percent X 2, 100 percent X 2). Then, in Columbia, clear with xylene (3-4 X) staining dish.[21] Finally, slides were mounted to capture an image using the 40X Cilika-Pro Digital microscopy Biospectrum, India.

PS special staining (Picrosirius red stain)

Dewax and hydrate the sections of paraffin. Stains nuclei with Weigert's hematoxylin for 8 minutes, and then washes the slides in running tap water for 10 minutes. After that stain in picro-sirius red for an h then wash two changes of acidified water. Any of the water from the slides is physically separated by intense shaking. Dehydrate in 100 % ethanol with three changes.[22] Clear in xylene and mount in a resinous medium to capture an image using the 40X Cilika-Pro Digital microscopy Biospectrum, India.

Masson's trichrome stain

Deparaffinized the blocks and rehydrated with 100% alcohol, 95% alcohol, 70% alcohol, and washed in distilled water. Re-fix in Bounin's solution for 1h at 56^{o}C for Formalin-fixed tissue to enhance staining efficiency, although this step is not required. And stain for 10 mins in Weighert's iron hematoxylin working solution. Rinse for 10 min in warm flowing tap water. Satin again for 10-15 mins in the Biebrich scarlet-acid fuchsin solution. Rinse again briefly in distilled water and differentiate from 2-5 minutes in 1% acetic acid solution. Finally, by 95% ethyl alcohol, absolute ethyl alcohol (these steps will wash off Biebrich scarlet- acid fuchsin staining), and clear in xylene, dehydrate very easily.[23] Mount with resinous mounting medium to capture an image using the 40X Cilika-Pro Digital microscopy Biospectrum, India.

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Results

Effect of aescin on glycoproteins (Mucins)

Figure 1 illustrates cell surface glycoconjugates expression in the buccal mucosa of control and experimental animals in each group. DMBA treated buccal mucosa of hamsters showed enhanced glycoconjugates expression. The expression of glycoconjugates in the buccal mucosa of DMBA painted hamsters was significantly reduced after oral administration of aescin (20, 40, and 80 mg/kg b.wt). The expression patterns of glycoconjugates were equivalent in Aescin alone treated and control hamsters.

Effect of aescin on collagen expression patterns

Figure 2 shows the collagen expression patterns in the buccal mucosa of control and experimental animals in each community. The buccal mucosa of hamsters treated with DMBA showed increased collagen expression. The gradual change of colour from red to brown was used to assign this. The collagen expression in the buccal mucosa of DMBA painted hamsters was significantly reduced after oral administration of aescin. Here the difference was noticed by endured red. Collagen expression patterns in aescin-alone treated and control hamsters were identical, although both groups were exposed to the red colour.

Effect of aescin on collagen fiber and muscle fiber.

Figure 3 shows the qualitatively examined histological tumour descriptions of control and aescin alone treated groups had normal cellular structural architecture, with augmented collagen fiber and muscle fibers. Collagen expression patterns in the buccal mucosa of control and experimental animals in each group were examined by Masson’s trichrome stain. The connective tissue is dyed blue, the cytoplasm is stained pink/red, and the nuclei are stained dark purple/red. Hyperkeratosis and differentiated muscle fibers where the collagen fiber content is thick were seen in the DMBA-treated classes. Mild keratosis and dispersed collagen fiber were observed in the oral administration of aescin at doses of 20, 40, and 80 mg/kg b.wt.

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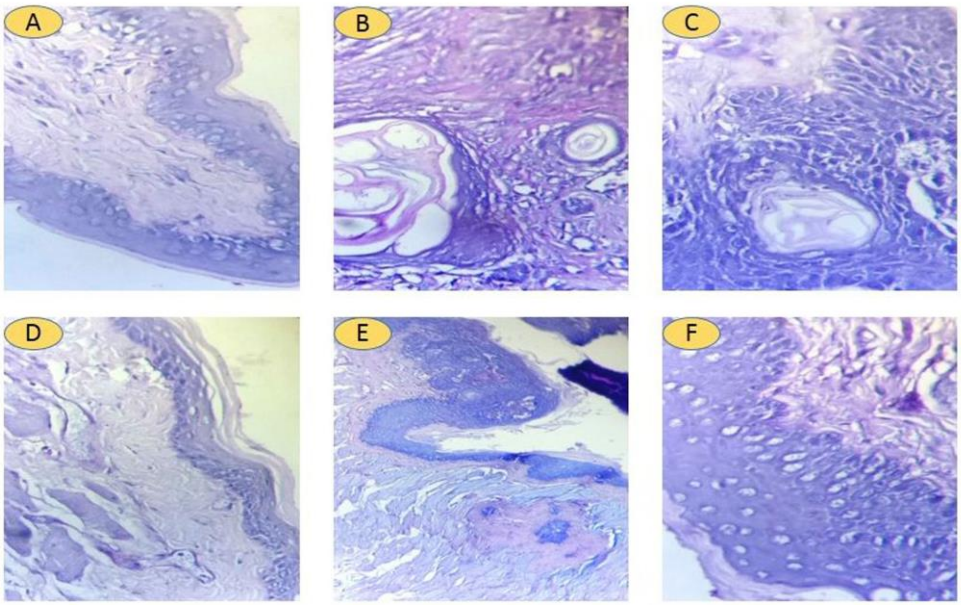

Figure 1: Control (A) and aescin treated alone (F) observed a normal level of glycoproteins using PAS stain. C, D, & E aescin treated (20, 40, and 80 mg/kg b.wt), DMBA induced oral squamous cell carcinoma showing moderately differentiated staining of glycoproteins around tumor island using at dose-dependent manner. DMBA induced oral squamous cell carcinoma (B) showing induced staining level of glycoproteins around tumor Island. Photomicrograph visualized under 40X Cilika-Pro Digital microscopy Biospectrum, India.

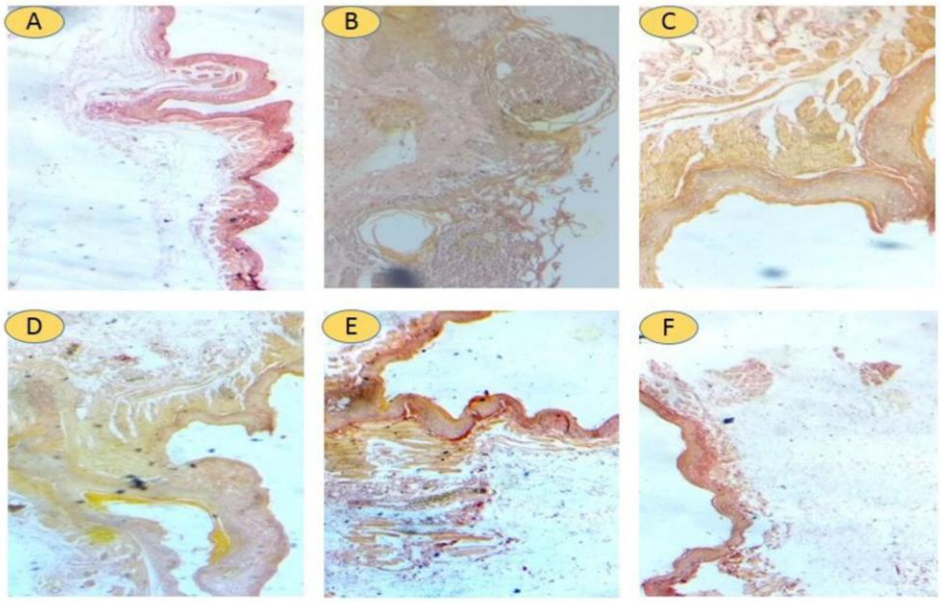

Figure 2: Control (A) and Aescin treated alone (F) showing thin normal collagen fibers using picrosirius red stain. C, D, & E were the aescin treated (20, 40, and 80 mg/kg b.wt), DMBA induced oral squamous cell carcinoma showing moderately differentiated oral squamous cell carcinoma showing thick mature collagen fibers. DMBA induced oral squamous cell carcinoma (B) showing

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thick mature collagen fibers using picrosirius red stain around tumor island. Photomicrograph visualized under 40X Cilika-Pro Digital microscopy Biospectrum, India.

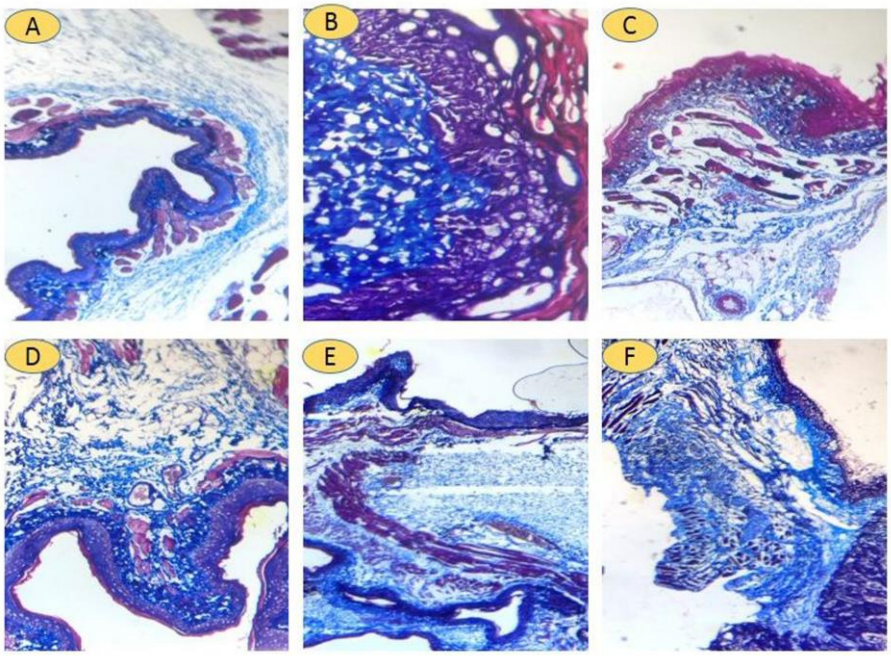

Figure 3: Photomicrographs of buccal mucosal specimens obtained from control (A) and aescin treated alone (F) show normal collagen deposition. (B) DMBA treated buccal mucosa in comparison with its corresponding control (A) this photomicrograph shows highly dense collagen deposition in the connective tissue, with red cytoplasmic stain dispersed among blue collagen bundles. Aescin-treated hamsters (C, D, &E) changes were observed with a reduced level of collagen deposition.

Discussion

OSCCs are extremely heterogeneous in terms of genetics and tumour biology, and they have poor clinical outcomes.[24] Chemotherapeutic agents that can stop tumors from growing are often linked to unpleasant side effects.[25] Aescin has anti-tumor properties in pancreatic cancer, colon cancer, gastric adenocarcinoma cells, and colorectal cancer (CRC) cancer.[26-29] In this research, we establish the effect of aescin on collagen and glycoproteins on DMBA induced oral carcinoma.[30]

The association between cancer cells and the surrounding extracellular matrix (ECM) is one of the most important aspects of tumour cell invasion and metastasis.[17] This interaction involves all extracellular matrix components is the most abundant type I collagen, which is predominantly produced by fibroblasts.[31] Through the evolution and progression of carcinoma, the collagen/collagenous tissue, often known as the stroma's “basic skeleton," undergoes significant changes in two distinct ways. Firstly, it can inhibit tumour progression by inhibiting the host immune response or by preventing tumour spread by inducing an

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abundant collagenous stroma (walling off effect). Second, proteolysis or changes in collagen composition may assist neoplastic cell mobilization into the stroma, facilitating subsequent invasion and metastasis**]**. Collagen can be used in clinical applications because it is closely linked to clinical outcomes, also used as a prognostic and recurrence indicator, in diagnosis, as a treatment resistance biomarker, in targeted therapy, and as a drug carrier. [14,30]

Mucins are the main organic components of mucus. Mucus is a slimy visco-elastic substance that coats all mucosal surfaces. Generally, these molecules were assumed to come from cells that secrete "mucus." Hence, in salivation, mucins are contributed by the submandibular, sublingual, and various small salivary organs which are strategically located in the oral cavity.[32] Mucins are large extracellular protein, which is heavily glycosylated with complex oligosaccharides that establish the selective molecular barrier at the epithelial surface and involve in morphogenetic signal transduction.[33] Mucin expression or glycosylation changes are linked to the development of cancer and affect cellular growth, tumour progression, invasion, and metastasis, as well as tumour cell survival and immune defense.[34] Changes in mucin expression and glycosylation have been linked to a variety of diseases, including carcinomas**.** Mucins are used as cancer diagnostic markers and are being investigated as a cancer therapeutic target. Mucins are overexpressed in various cancer includes non–small-cell lung cancer (NSCLC), colon cancer, breast carcinoma, oral cancer, according to this several studies, mucins may be used as biomarkers for detecting and tracking lung cancer progression.[35-38] Kohli M *et al.*, 2019[38] suggest that mucin plays a vital role in the pathogenesis of OSCC and can be regarded as a novel marker for OSCC. The understanding of the molecular mechanisms of aescin on Collagen and glycoproteins may provide novel insights for the development of effective treatments for OSCC. According to previous studies, aescin was effective in decreasing the levels of collagen in the liver**.** Gui-Li H *et al.*, 2015[39] revealed that aescin reversers the expression of P-glycoprotein in cholangiocarcinoma. We observed, the status of cell surface abnormalities by evaluating the expression of the glycoprotein of the mucins (Figure.1), collagen fiber, and muscle fibers (Figure. 2&3) via histochemical staining techniques. Similar to the previous studies, our research findings show that aescin at doses of 20, 40, and 80 mg/kg b.wt., which restored nearly normal collagen architecture (Figure. 2&3) and mucin expression (Figure.1) in DMBA induced OSCC.

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Conclusion

In conclusion, we suggest that aescin having potent anticancer activity against DMBA induced oral carcinogenesis in Hamster buccal pouch (HBP) model possibly protects through the restoration of collagen and glycoproteins to normal and also thereby reverse the status of cell surface architectural abnormalities. According to our findings, aescin protects against collagen breakdown during DMBA-induced oral carcinogenesis and significantly reduced cell surface abnormalities during DMBA-induced OSSC. Overall the findings of this study conclude that new insight of aescin might be developed as a potential chemopreventive agent for OSCC.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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