

Adhatoda vasica Leaves Protect Cell Surface Glycoconjugates Abnormalities During DMBA Induced Hamster Buccal Pouch Carcinogenesis.

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ABSTRACT

Glycoproteins play an integral role in several biological functions including cell-cell interaction. Altered glycoproteins in the cell membrane or surface leads to malignant transformation. The main objective of the present study is to explore the protective effect of *Adhatoda vasica* on cell surface abnormalities during DMBA induced hamster buccal pouch carcinogenesis. Hamsters treated with DMBA alone for 14 weeks (thrice a week) in the buccal pouches resulted in well developed and well differentiated squamous cell carcinoma. Increased glycoprotein content was noticed in both tumor tissues and circulation of hamsters treated with DMBA alone. Oral administration of *Adhatoda vasica* ethanolic leaf extract (AVELet) at a dose of 100 mg/kg bw to hamsters treated with DMBA prevented the oral tumor formation as well as protected the DMBA- induced cell surface abnormalities. The present study thus concludes that *Adhatoda vasica* has the ability to protect the cell surface abnormalities induced by the carcinogen, DMBA, during oral carcinogenesis.

Key words: *Adhatoda vasica*, glycoconjugates, DMBA, oral cancer.

INTRODUCTION

Oral cancer causes significant morbidity and mortality worldwide and imposes burdens on the survival as well as life quality of the patients. Though this form of cancers affects 2-3 % of western populations, the incidence is very high in developing countries such as India, where it accounts for 40-50% of all cancers¹. Tobacco and betel quid chewing along with alcohol abuse are pointed out as major risk factors of oral carcinogenesis². Early diagnosis of oral cancer could only help to prevent or improve the life quality of the patients, despite recent advancements in chemotherapy and radiotherapy³. Hence, investigation of novel agents from medicinal plants with low toxicity or less side effects are still warranted not only to treat oral cancer at an early stage but also to improve the survival outcome of the patients.

Tumor cells exhibit several biochemical abnormalities as compared to their normal cellular counterpart⁴. One of the most significant abnormalities noticed in tumor cell surface is in the status of glycoproteins. Glycoproteins, proteins conjugated with oligosaccharide chain, play a pivotal role in several cellular mechanisms including cell adhesion and cell- cell communication⁵. Normal cells are converted into malignant ones if glycoproteins are abnormally expressed on the cell surface. Sialic acid and fucose, the two important glycoconjugates, play crucial role in neoplastic transformation⁶. Profound scientific studies clearly demonstrated abnormal levels of

glycoproteins, especially sialic acid and fucose, in both human and experimental carcinogenesis⁷⁻⁸.

Medicinal plants serve as an important resource for the treatment of several disorders including cancer. So far, around thirty anticancer drugs have been isolated from medicinal plants and are currently used in cancer treatment. Though several medicinal plants were tested for their anticancer efficacy, the anticancer potential of several other plants remains to be elucidated. *Adhatoda vasica*, is one such plant possesses a spectrum of bioactive principles to treat various diseases. The Indian traditional system of medicine like Ayurveda strongly recommends *Adhatoda vasica* to treat various disorders including cancer⁹. Experimental studies documented its hepatoprotective, anti-inflammatory, antimicrobial and anticancer properties¹⁰⁻¹³. Phytochemical analysis revealed the presence of quinazoline, vasicine, vasicinone, deoxyvasicine and phenolic compounds¹⁴. The major aim of the present study is to assess the protective effect of *Adhatoda vasica* on the status of cell surface glycoconjugates in DMBA induced oral carcinogenesis.

MATERIALS AND METHODS

Preparation Of Plant Extract: The *Adhatoda vasica* leaves were collected in and around Chidambaram, Cuddalore district, Tamilnadu, India, in August to September at the end of flowering season. The taxonomic identification of the plant was compared with the existing herbarium in the Botany department of Annamalai University. Five hundred

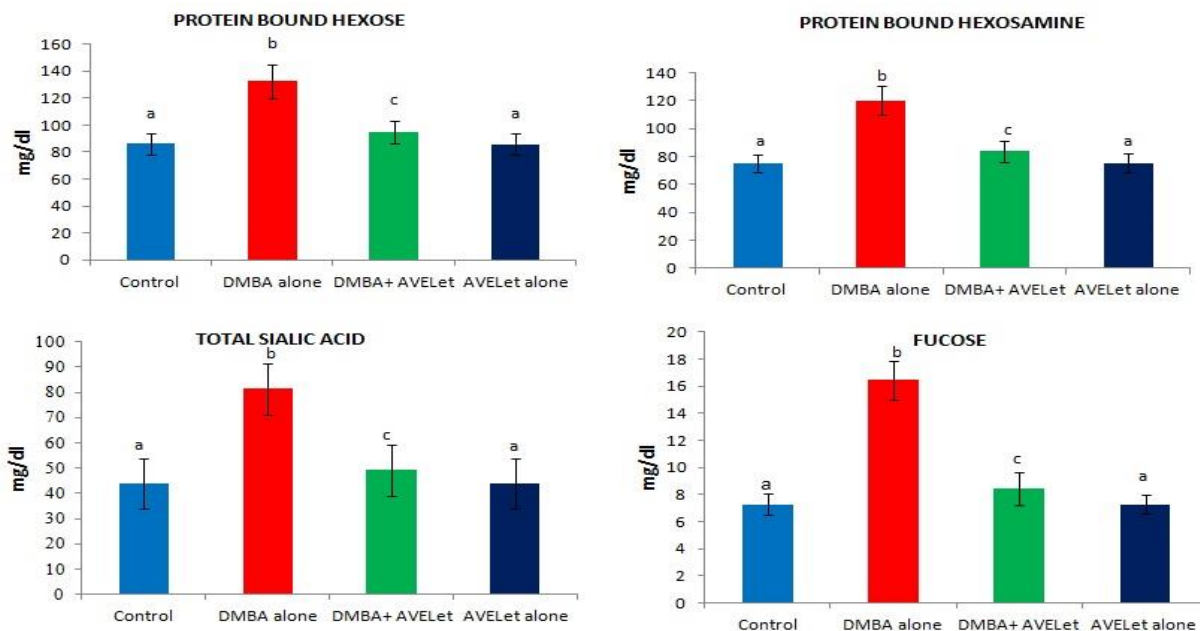


Fig. 1: The levels of glycoconjugates (protein-bound hexose, hexosamine, total sialic acid and fucose) in the plasma of control and experimental hamsters in each group.

Values are expressed as mean \pm Standard deviation (S.D) for ten hamsters in each group. Values that do not share a common superscript between groups differ significantly at $p < 0.05$ (DMRT).

grams of dried, finely powdered *Adhatoda vasica* leaves were soaked with 1500 ml of 95% ethanol overnight. The residue obtained after filtration was again resuspended in equal volume of 95% ethanol for 48h and filtered again. The above two filtrates were mixed and the solvents were evaporated in a rotavapour at 40-50% under reduced pressure. A semisolid material (7%) obtained was stored at -4°C for further experimental use. A known volume of the residual extract is suspended in distilled water and orally administered to the animals (100 mg / kg bw) by gastric intubation using force feeding tube during the experimental period. The above dose was selected on the basis of dose dependent studies.

Experimental Design: Forty male golden Syrian hamsters, 8 weeks old, weighing 80-120g, were obtained from National Institute of Nutrition, Hyderabad, India and maintained in Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University. The institutional animal ethics committee [Reg.no:160/1999/CPCSEA], Annamalai University, Annamalaiagar, approved the experimental design. The hamsters were divided into four groups of 10 hamsters in each. Group I hamsters served as the vehicle-treated control and painted with liquid paraffin alone three times a week for 14 weeks on their left buccal pouches. Groups II and III hamsters were painted with 0.5% DMBA in liquid paraffin three times a week for 14 weeks on their left buccal pouches. Group II hamsters received no further treatment. Group III hamsters received oral administration of *Adhatoda vasica* ethanolic leaf extract (AVELet) at a dose of 100 mg / kg bw, starting one week before the exposure to the carcinogen and was continued on days alternative to DMBA painting for 14 weeks (pre-initiation phase). Group IV hamsters received oral administration of

AVELet (100 mg/ kg bw) alone throughout the experimental period. The experiment was terminated at the end of the 16th week (pre-initiation phase) and all hamsters were sacrificed by cervical dislocation. Biochemical studies were conducted in the plasma and buccal mucosa of control and experimental hamsters in each group.

Analysis of Glycoconjugates: The precipitate obtained after treating the plasma with 95% ethanol was used for the estimation of the protein-bound hexose¹⁵, hexosamine¹⁶, total sialic acid¹⁷, and fucose¹⁸. The defatted tissues were treated with 0.1N H_2SO_4 and then hydrolyzed at 80°C for 1 hour. It was cooled and the aliquot was used for the sialic acid estimation. To the remaining solution, 0.1N sodium hydroxide was added and it kept in an ice bath for 1 hour and used for the estimation of protein-bound hexose and fucose.

Statistical Analysis: The data are expressed as mean \pm SD. Statistical comparisons were performed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT). The results were considered statistically significant at $P \leq 0.05$.

RESULTS

Figures 1 and 2 depict the levels of glycoconjugates (protein-bound hexose, hexosamine, total sialic acid and fucose) in the plasma and buccal mucosa (protein-bound hexose, total sialic acid and fucose) of control and experimental hamsters in each group respectively. The levels of glycoconjugates in the plasma and buccal mucosa were significantly increased in hamsters treated with DMBA as compared to control hamsters. Oral administration of *Adhatoda vasica* ethanolic leaf extract (AVELet) at a dose of 100 mg/kg bw to DMBA-treated

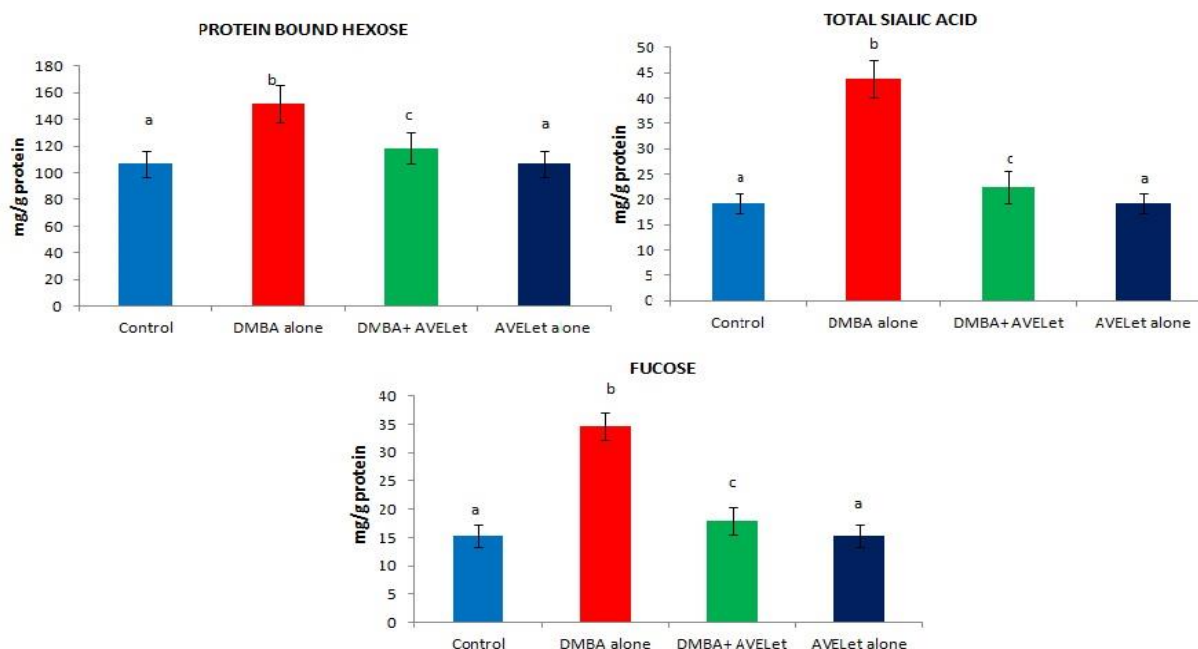


Fig. 2: The levels of glycoconjugates (protein-bound hexose, total sialic acid and fucose) in the buccal mucosa of control and experimental hamsters in each group.

Values are expressed as mean \pm Standard deviation (S.D) for ten hamsters in each group. Values that do not share a common superscript between groups differ significantly at $p < 0.05$ (DMRT).

hamsters brought back the levels of the above-mentioned glycoconjugates to near normal levels. Hamsters treated with *AVELet* alone showed no significant difference in the levels of plasma and buccal mucosa glycoconjugates as compared to control hamsters.

DISCUSSION

A large number of cancer researchers focused the significance of glycoproteins as tumor specific markers¹⁹⁻²¹. Glycoconjugates have a vital role in many biological processes such as cell-cell recognition, cell growth and differentiation, and cell-matrix interaction²². Abnormal expression of glycoconjugates facilitates invasion and metastasis²³. It has been reported that tumor cells secrete or express new glycoproteins moiety on their cell surface as well as glycoproteins are secreted into circulation from the destructed tumor tissues²⁴. Atypical glycosylation has been recognized as a characteristic feature of oral malignant transformation²⁵. Glycoconjugates status could help to assess the prognosis as well as monitoring the treatment strategy²⁶. Cell surface glycoconjugates are abnormally increased if the cells are exposed to potent carcinogens.

DMBA induced cell surface abnormalities in relation to the expression of glycoconjugates are well documented²⁷. Abnormal levels of glycoproteins in plasma and tumor tissues were reported in several cancerous conditions including oral cancer²⁸⁻³⁰. The levels of glycoconjugates are increased in a stepwise manner from stage I to stage IV of oral malignancies³¹. Tumor cells abnormally express sialic acid, a nine carbon sugar commonly known as N-acetyl neuraminic acid³². Extensive studies pointed out that tumor cells have two fold increase in sialic acid concentrations as compared to their normal cellular

counterpart^{33,34}. Fucose, the terminal moiety of glycoconjugates, is a five carbon sugar and plays a crucial role in malignant transformation. Increased concentration of fucose has been reported in plasma and tumor tissues of both human and experimental oral carcinogenesis^{35,36}. A large number of cancer researchers pointed out that increased turnover of sialic acid and fucose in tumor tissues with subsequent leakage into circulation could account for increased levels of plasma fucose and sialic acid^{37,38}. Increased levels of glycoconjugates in tumor tissues could be due to neosynthesis of glycoproteins in cancerous conditions. Increased levels of plasma glycoproteins are probably due to secretion from the destructed tumor tissues³⁹. Increased levels of glycoconjugates may also be due to abnormal glycosylation, fucosylation and sialylation in the tumor tissues⁴⁰.

In the present study, we analysed the status of glycoproteins in the tumor tissues as well as in the circulation in the oral tumors bearing animals due to the fact that glycoproteins play significant role in cellular proliferation as well as in tumor invasion and metastasis. In the present study, oral administration of *Adhatoda vasica* ethanolic leaf extract at a dose of 100 mg/kg bw significantly decreased the levels of glycoconjugates in the buccal mucosa and plasma of tumor bearing hamsters. The present results thus indicate that *Adhatoda vasica* leaves has the potential to inhibit the activities of enzymes that are involved in the process of glycosylation, fucosylation and sialylation. The protective potential of *Adhatoda vasica* further implies that it significantly prevented the destruction of tissue damage by the carcinogen, DMBA. The present study thus demonstrates the protective efficacy of *Adhatoda vasica* on cell surface

glycoconjugates abnormalities during DMBA induced oral carcinogenesis. Further studies are warranted to investigate the effect of *Adhatoda vasica* on the activities of enzymes involved in glycosylation, fucosylation and sialylation process during DMBA induced oral carcinogenesis.

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