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ENICOSTEMMA LITTORALE PROTECTS CELL SURFACE ABNORMALITIES DURING DMBA-INDUCED HAMSTER BUCCAL POUCH CARCINOGENESIS

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ABSTRACT

Glycoconjugates [protein bound hexose, protein bound hexosamine, total sialic acid and fucose] play a pivotal role in neoplastic transformation if they are abnormally expressed. The protective effect of *Enicostemma littorale* leaves on cell surface abnormalities was analyzed by measuring the status of the above said glycoconjugates during 7,12-dimethylbenz(a) anthracene (DMBA) induced hamster buccal pouch carcinogenesis. Tumors were developed in the buccal mucosa of hamsters by painting with DMBA thrice a week for 14 weeks. Elevated levels of glycoconjugates were observed in both plasma and tumor tissues of hamsters treated with DMBA alone. Oral administration of *Enicostemma littorale* leaf extract at a dose of 250 mg / kg bw to hamsters treated with DMBA significantly brought back the levels of glycoconjugates to near normal range. *Enicostemma littorale* leaves, thus have the potential to prevent the abnormalities in the glycoconjugates status occurring in DMBA induced oral carcinogenesis.

KEY WORDS: **Oral cancer, DMBA, Enicostemma littorale, Glycoconjugates.**

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INTRODUCTION

Glycoproteins, proteins that contain oligosaccharide chains, play crucial functions in cell-cell interactions[1]. Glucose, galactose, mannose, fucose, N-acetylgalactosamine, N-acetylglucosamine, and N-acetylneuraminic acid are the predominant sugars in glycoproteins [2]. Glycoproteins exist in the cells, both in soluble and membrane-bound forms, as well as the extracellular matrix and in extracellular fluids. Glycosylation of proteins is essential for protein folding, targeting and ligand binding [3]. The abnormal glycosylation pattern may lead to neoplastic transformation [4]. They serve as an integral part of the extracellular matrix and play pivotal role in several physiological and biological processes. Glycoconjugates serve as an important biomarker for the diagnosis and prognosis of several cancers [5]. Glycoconjugates levels were found to be increased in the tumor tissues of several malignant cancers including oral carcinoma [6]. Oral malignant transformation is usually associated with atypical glycosylation of cell surface glycoconjugates [7]. Malignant neoplasms of the oral cavity account for 40-50% of all cancers in India and this form of cancers arises mainly due to tobacco and betel quid chewing, smoking and heavy consumption of alcohol. Golden Syrian hamsters are used as an ideal model for oral cancer research due to its pocket like anatomy [buccal pouch], which retains the carcinogens for longer time during the topical application of carcinogen treatment. DMBA, a potent organ specific carcinogen, can induce oral carcinoma in the buccal pouch of hamsters, which exhibit biochemical, molecular and histopathological similarities to human oral cancer [8]. DMBA mediates carcinogenesis through chronic inflammation, multiple mutations and genetic alterations and via excessive generation of reactive oxygen species occurring during metabolic activation of DMBA. DMBA induced hamster buccal pouch carcinogenesis is therefore commonly used to study the biochemical and molecular mechanisms of oral carcinogenesis as well as to test the chemopreventive efficacy of the natural products and its active constituents [9]. *Enicostemma littorale*, a perennial herb belongs to Gentianaceae family, is found throughout India especially in the coastal regions. It is commonly known as Indian genitian in English and traditionally used to treat several kinds of illness, including malaria, diabetes, inflammation and ulcer [10,11]. Profound experimental studies reported its antioxidant,antitumor and hypolipidemic potential. Phytochemical analysis showed the presence of various bioactive constituents, which include tannins, anthroquinones and flavanoids [12,13]. In vitro studies have documented its cytotoxic potential in various cancer cell lines.The present study is designed to explore the protective effect of *Enicostemma littorale* leaves on DMBA induced cell surface abnormalities in the buccal pouch of golden Syrian hamsters.

MATERIALS AND METHODS

Animals

Golden Syrian hamsters weighing 80–120 g were purchased from National Institute of Nutrition, Hyderabad, India and maintained in the Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University.

Plant material

Enicostemma littorale leaves, collected in and around Chidambaram, Cuddalore District, Tamilnadu, India,are authenticated by the Botanist, Department of Botany, Annamalai University.

Preparation of the plant extract

The two filterates obtained after soaking 500 gm of *Enicostemma littorale* leaves subsequently with 1500 ml of 95% ethanol for 48h were mixed and allowed to evaporate in a rotavapour under reduced pressure. A semisolid material (9%) obtained was stored in 4º C until used.

EXPERIMENTAL DESIGN

Annamalai University animal ethics committee [Reg No: 160 / 1999 /CPCSEA], approved the experimental design. Forty male golden Syrian hamsters were categorized into four groups of ten animals in each. Group I hamsters were

treated with liquid paraffin alone three times a week for 14 weeks on their left buccal pouches. Hamsters in groups II and III were treated with 0.5% DMBA in liquid paraffin three times a week for 14 weeks on their left buccal pouches. Group II hamsters were received only DMBA treatment. Group III hamsters were, however orally administered with ethanolic extract of *Enicostemma littorale* leaves (EIELet 250 mg / Kg bw) on alternative days of DMBA treatment. Group IV hamsters were received EIELet alone orally throughout the experimental period. All the experimental hamsters were sacrificed by cervical dislocation at 16th week. Glycoconjugates analysis was conducted in the plasma and buccal mucosa of control and experimental animals in each group. The protein bound hexose, hexosamine, total sialic acid and fucose were estimated by the methods of Niebes [14] Wagner et al [15] Warren et al [16] and Dische and Shettles [17] respectively.

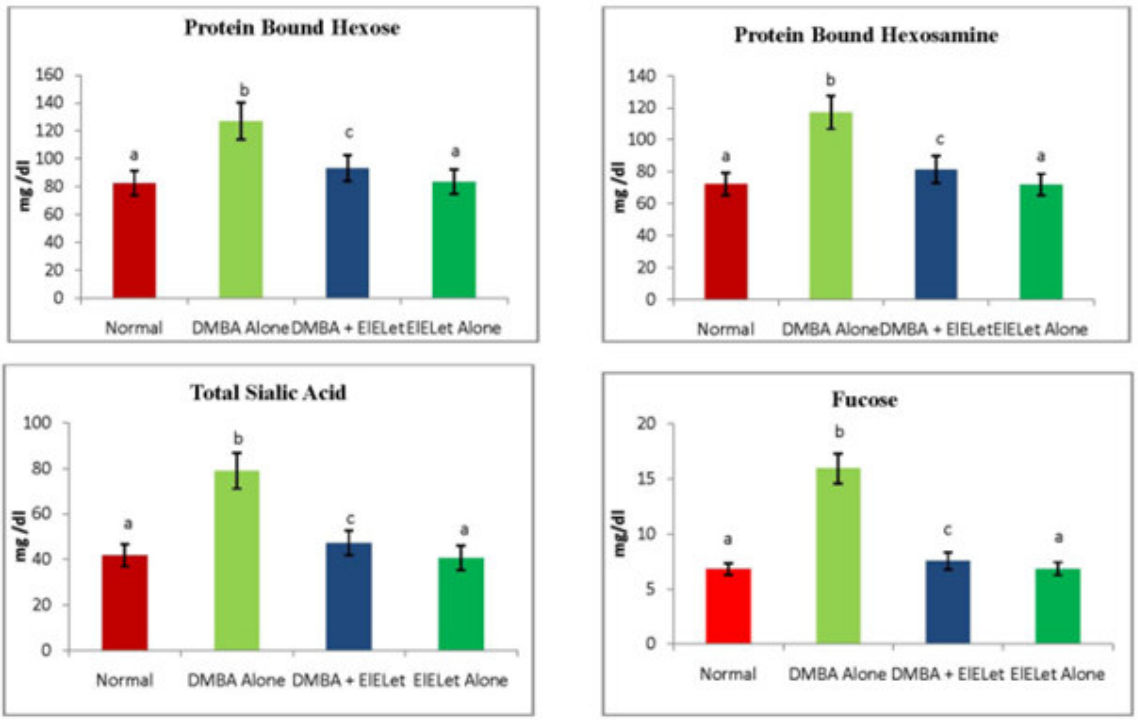
Statistical analysis

Statistical analysis was done using One-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT). The difference in glycoconjugates levels between two groups were considered statistically significant if the p values were 0.05 or less.

RESULTS

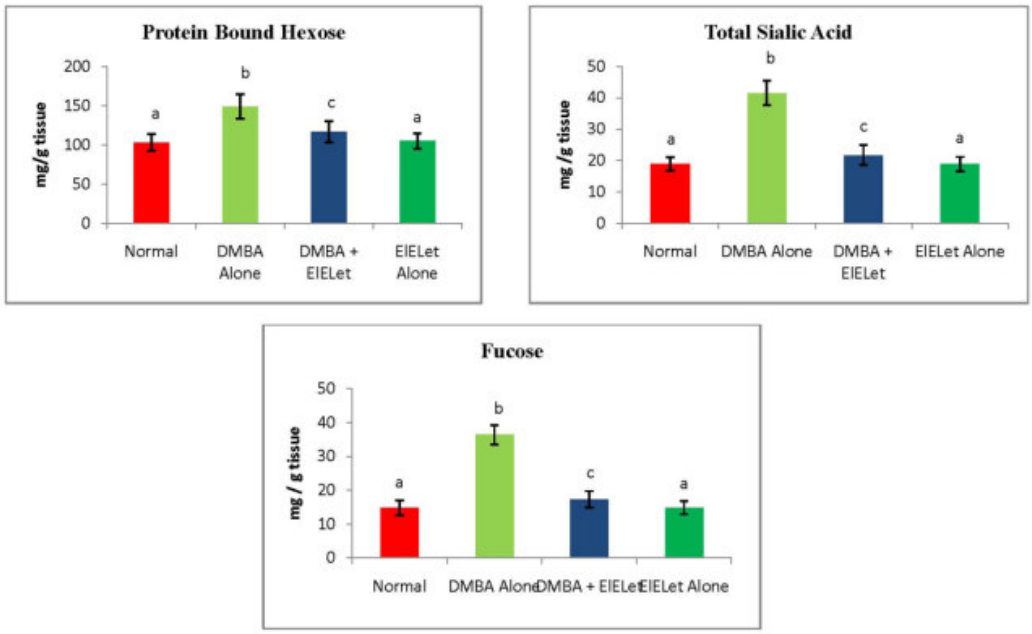
The plasma and buccal mucosa glycoconjugates status is shown in figures 1 and 2 respectively. The levels of glycoconjugates in the plasma and buccal mucosa were significantly increased in hamsters treated with DMBA alone (tumor bearing animals) as compared to control hamsters. EIELet administered orally at a dose of 250 mg / kg bw to hamsters treated with DMBA restored the levels of plasma and buccal mucosa glycoconjugates to near normal range. Plasma and buccal mucosa glycoconjugates in EIELet alone treated and control hamsters are statistically non – significant

Figure 1 shows the levels of glycoconjugates in plasma [Protein Bound Hexose, Hexosamine, Total Sialic Acid and Fucose] of control and experimental hamsters in each group.



Values are expressed as mean± SD (n=10). Values that are not sharing a common superscript differ significantly at p<0.05(DMRT).

Figure 2 shows the levels of glycoconjugates in buccal mucosa [Protein Bound Hexose, Total Sialic Acid and Fucose] of control and experimental hamsters in each group.



Values are expressed as mean± SD (n=10). Values that are not sharing a common superscript differ significantly at $p<0.05$(DMRT).

DISCUSSION

Glycans of glycoproteins, proteoglycans and glycolipids play pivotal role in cell adhesion, cell differentiation, cell – cell communication, metastasis and immune responses [18]. Glycoconjugates receive greater attention as cancer biomarkers due to the fact that several tumour cells abnormally express the glycoproteins and glycoproteins play a pivotal role in tumor progression and metastasis [19]. Glycoproteins, a major constituent of cell membranes, are abnormally expressed in precancerous and cancerous conditions. Neoplastic transformation occurs due to alterations in cell surface components, especially glycoproteins [20]. Extensive studies on various cancer research documented over expression of glycoproteins in the circulation and tumor tissues [21,22]. Manoharan et al [23] reported that plasma glycoproteins levels are increased from stage I to IV of oral cancer patients. It has been reported that abnormal levels of plasma or serum glycoproteins are due to the secretion of glycoproteins from the destructed tumor tissues [24]. Increased secretion and shedding from malignant tumors could account for elevated plasma protein bound hexose and hexosamine. Though the serum concentration of L-fucose is found to be low under normal physiological conditions, its levels are abundantly increased in cancerous condition. Increased serum fucose level was reported in head and neck cancers [25]. Higher concentrations of plasma fucose and sialic acids are due to increased turnover in the tumor cells with subsequent linkage into plasma [26]. Sialic acid, a nine carbon sugar, exists as end moieties in the glycoconjugates and plays a pivotal role in several biological functions including cellular invasiveness and adhesiveness [27]. It has been demonstrated that abnormalities in sialylation in fucosylation could result in malignant transformation [28]. Extensive studies documented the anticancer potential of various medicinal plants by either *in vitro* or *in vivo* approach[29.30]. In the present study, we noticed 100% tumor formation in the buccal mucosa of hamsters treated with DMBA alone and the tumors were histopathologically confirmed as well differentiated squamous cell carcinoma by the

oral pathologist. Oral administration of *Enicostemma littorale* at a dose of 250 mg / kg bw not only prevented the formation of tumors in hamsters treated with DMBA but also brought back the status of glycoconjugates to near normal range. However, we observed mild precancerous lesions such as hyperplasia and dysplasia in DMBA+ *Enicostemma littorale* treated hamsters. Our results suggest that *Enicostemma littorale* has protected the abnormalities of cell surface glycoconjugates during DMBA induced hamster buccal pouch carcinogenesis. The protective effect of *Enicostemma littorale* is either due to its preventive efficacy on the destruction of buccal mucosa tissues or due to its inhibitory effect on enzymes involved in the glycosylation, fucosylation or sialylation process. Further studies are in progress to investigate the effect of *Enicostemma littorale* leaves on the enzyme pattern involved in glycosylation, fucosylation and sialylation process processes.

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