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Research Article

## Cissus quadrangularis protects cell surface glycoconjugates during 7,12-dimethylbenz(a)anthracene induced oral carcinogenesis

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### ABSTRACT

Glycoproteins play a pivotal role in the maintenance of cellular integrity and thus alterations in the status of glycoproteins could lead to several pathological conditions including malignant transformation. The aim of the present study is to explore the effect of the ethanolic extract of the *Cissus quadrangularis* leaves on the plasma and buccal mucosa glycoproteins in 7,12-dimethylbenz(a)anthracene (DMBA) induced hamster buccal pouch carcinogenesis. Oral carcinoma was induced in the hamsters' buccal pouch using the site specific carcinogen, DMBA (3 times a week for 14 weeks, topical application). Plasma and buccal mucosa glycoproteins were found to be enhanced in both the plasma and buccal mucosa of hamsters treated with DMBA alone (tumor bearing hamsters). The status of glycoproteins was found to be reverted in hamsters treated with DMBA + *Cissus quadrangularis* leaves. The present results thus reveal the defensive role of the *Cissus quadrangularis* leaves in preserving the cellular integrity during DMBA induced oral carcinogenesis.

**Keywords:** glycoproteins; oral cancer; DMBA; *Cissus quadrangularis*.

### INTRODUCTION

Glycoproteins perform multiple and complex functions in several biological processes. Glycoproteins contain aminosugars (Glucosamine and galactosamine), hexoses (glucose and galactose), sialic acid, and fucose in their carbohydrate moiety. Glycoproteins are utilized as a biomarker for not only the early detection of carcinogenesis but also in the prognosis of cancer treatment (Stowell *et al.*, 2015). Protein glycosylation is a major post-translational modification observed in the mammalian cells and any abnormalities in this mechanism could lead to various pathological diseases including cancer. The aberrant glycosylation pattern in the mammalian cells is a key event that accompanies neoplastic transformation (Pinho and Reis, 2015; Stowell *et al.*, 2015). Extensive studies pointed out abnormal protein glycosylation as a major phenotypic alterations in carcinogenesis (Pinho and Reis, 2015). The abnormal glycosylation pattern in the cells or tissues have been documented in various cancers (Chauhan and Lahiri, 2016; Pan *et al.*, 2016). Extensive literatures demonstrated the

abnormal levels of both N- linked and O-linked glycoproteins in cancerous conditions (Yang *et al.*, 2017; Manoharan *et al.*, 2011).

Chemotherapy, in combination with surgery and/or radiotherapy, has been considered as a preferred treatment modality for various cancers. However, due to various side effects of these therapeutic efficacies, intensive research is continuing to search an anticancer agent with low toxicity and fewer side effects. Natural products serve as a foundation of cancer chemotherapy for the last four decades and thus search for safe anticancer bioactive constituent is essential for new drug development in cancer research. In the Indian system of traditional medicine, several plants are documented for the treatment various human pathological diseases.

*Cissus quadrangularis*, a perennial rambling shrub, is commonly known as "Hadjora" in Hindi and Perandai in Tamil. This plant is documented as one of the valuable medicinal plants in the Indian traditional systems of medicine for the treatment of skin diseases, chronic ulcers, tumors, osteoarthritis, rheumatoid arthritis, and leprosy. It is found throughout India, Sri Lanka, Africa, Arabia, and Southeast Asia. Phytochemical analysis of *Cissus quadrangularis* indicates the presence of carotene, terpenoids,  $\beta$ -sitosterol, tannins, stilbenes, saponins,  $\delta$ -amyrin,  $\delta$ -amyrone quercetin and rutin and calcium (Sen and Dask, 2012). Nearly thirty potent bioactive constituents have been investigated in the aerial parts of *Cissus quadrangularis*. It has been reported that *Cissus quadrangularis* explored its cytotoxic potential

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through ROS generation and G1 phase cell cycle arrest in HeLa cell lines (Sheikh *et al.*, 2015; Dwivedi *et al.*, 2013). ROS-induced apoptosis has been proposed as a major mechanism for the anticancer potential of *Cissus quadrangularis* in cervical cancer cell lines (Sheikh *et al.*, 2015). The anti-lipid peroxidative potential of *Cissus quadrangularis* leaves has been shown in the EAC cell lines (Kumar *et al.*, 2014). The tumor preventive efficacy of *Cissus quadrangularis* has been demonstrated in Dalton's Ascitic lymphoma cell lines (Nalini *et al.*, 2011). It has been reported that *Cissus quadrangularis* induced apoptosis in A431 skin cancer cells through modulating Bcl-2/Bax ratio (Bhujade *et al.*, 2013). The antibacterial, antiviral hepatoprotective, gastroprotective effect, bone healing and anti-inflammatory potential of *Cissus quadrangularis* have also been reported (Sen and Dask, 2012, Swamy *et al.*, 2012). The present study is designed to investigate and scientifically validate the tumor protective efficacy of the ethanolic extract of *Cissus quadrangularis* leaves on cell surface glycoconjugates status in DMBA induced oral carcinogenesis.

## MATERIALS AND METHODS

### Preparation of the plant extract

500 grams of *Cissus quadrangularis* leaves were shade dried, finely powdered and then soaked in 1500 ml of 95% ethanol overnight. The filtrate was separated and was kept in a flask. The remaining residue was again soaked in 1500 ml 95% ethanol for further 48 h and filtered. The two filtrates were mixed, and the solvents were evaporated using a rotavapor at 40-50% under reduced pressure. The obtained semisolid material (9%) was stored at 4°C until further use. For the experimental study, residual extract at a dose of 250mg/kg body weight was suspended in distilled water and was orally administered to the animals by gastric intubation using force feeding tube.

### Experimental protocol

Experimental golden Syrian hamsters were divided into five groups and each group consists of six animals. The hamsters were maintained in the Animal House of Muthayammal college of Arts and Science, Rasipuram as per ethical committee principles. Oral tumors were developed in the buccal pouch of the animals using DMBA, the organ specific carcinogen. All the animals were permitted to obtain food and water *ad libitum*. The experimental design followed in the present study was as follows.

The experimental hamsters received topical application of liquid paraffin alone and 0.5% DMBA in liquid paraffin on their left buccal pouches three times a week for 14 weeks was categorized as group I and group II respectively. The hamsters that were received topical application of DMBA and oral administration of the ethanolic extract of *Cissus quadrangularis* leaves (250mg/kg bw) on alternate days for 14 weeks was categorized as group III. The hamsters that were received topical application

of DMBA, three times a week for 10 weeks, and the ethanolic extract of *Cissus quadrangularis* leaves (250mg/kg bw) orally three times a week from 11<sup>th</sup> to 16<sup>th</sup> week was categorized as group IV. The experimental hamsters that received the oral administration of the ethanolic extract of *Cissus quadrangularis* leaves (250mg/kg bw) alone, three times a week, throughout the experimental period were categorized as group V. The biochemical and histopathological studies were carried out in the blood and tissue samples of the sacrificed animals at the end of the experimental period.

### Biochemical estimations

The glycoproteins levels in the plasma and tissues of the experimental animals were analyzed using specific and sensitive colorimetric procedures. The protein bound hexose in the plasma and defatted buccal mucosa tissues were measured by the method of Niebes (1972). The protein bound hexosamine in the plasma was determined by the method of Wagner (1979). The total sialic acid in the plasma and defatted buccal mucosa tissues were estimated by the method of Warren (1959). Fucose in the plasma and buccal mucosa tissue were estimated by the method of Dische and Shettles (1948).

### Statistical analysis

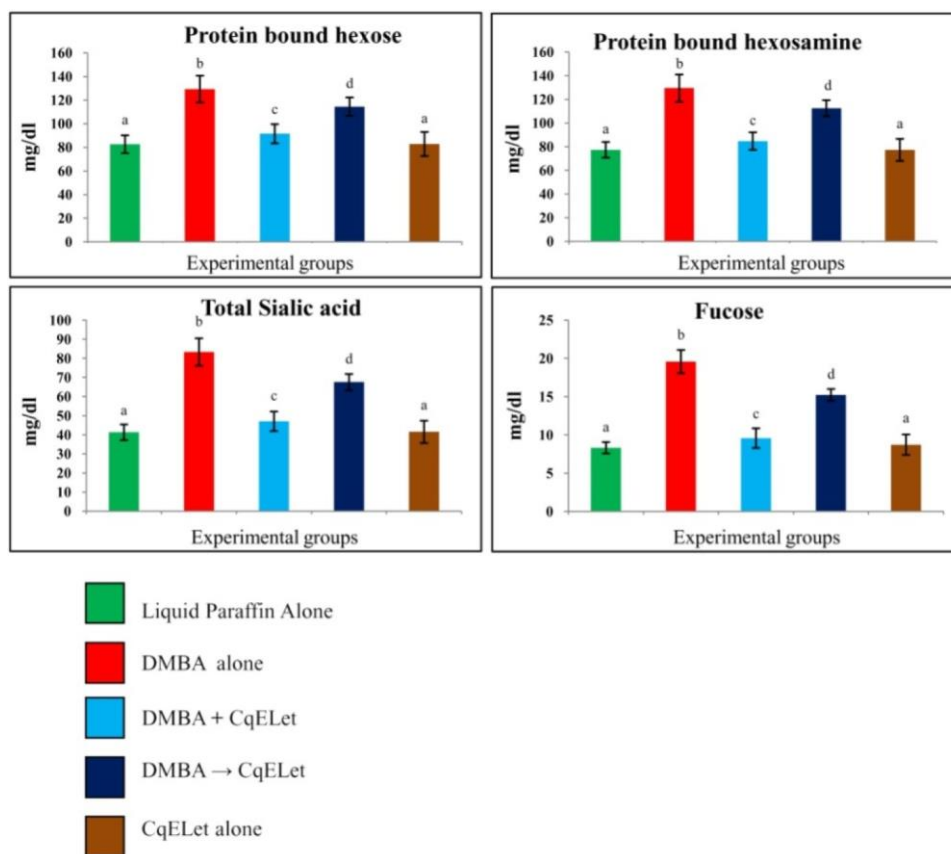
The biochemical variables are expressed as mean  $\pm$  SD. The one way of analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) was employed to compare the statistical significance between the groups. The two groups are considered statistically significant only if the p values were found to be less than 0.05.

## RESULTS

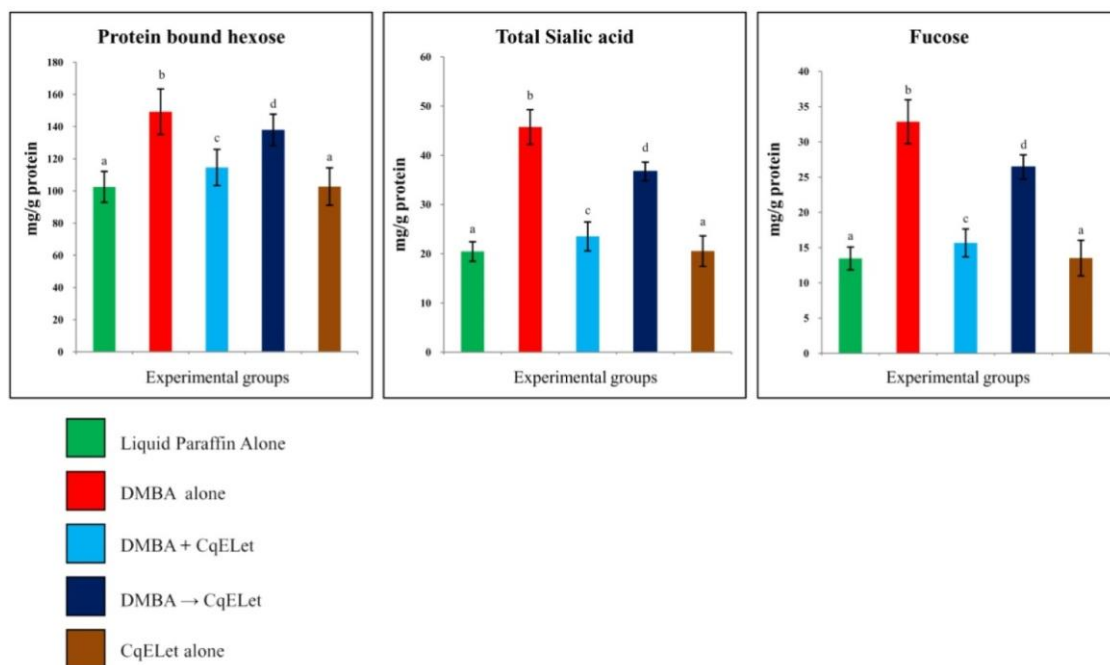
Glycoproteins status of plasma (protein bound hexose, hexosamine, sialic acid and fucose) and buccal mucosa (protein bound hexose, sialic acid and fucose) in control and experimental hamsters in each group are depicted in figures 1 and 2 respectively. The plasma and buccal mucosa from hamsters treated with DMBA alone showed increased levels of glycoproteins as compared to control hamsters. The administration of ethanolic extract of *Cissus quadrangularis* leaves orally to DMBA treated hamsters significantly reduced the levels of glycoproteins. Hamsters treated with the ethanolic extract of *Cissus quadrangularis* leaves alone and the control hamsters revealed similar patterns of glycoproteins in both plasma and the buccal mucosa. *Cissus quadrangularis* significantly improved status of plasma and buccal mucosa glycoproteins in the post-initiation phase as well.

## DISCUSSION

Glycoproteins, the major cell membrane constituents, serve a major role in various biological processes including cell-cell communication and cell adhesion. Several authors utilized the status of glycoproteins as valuable biomarkers to assess the tumor staging and to monitor



**Figure 1: The levels of glycoconju gates in the plasma of control and experimental hamsters**  
 Values are expressed as mean± SD (n=6). Values that are not sharing a common superscript differ significantly at P<0.05 (DMRT).



**Figure 2: The levels of glycoconjugates in the buccal mucosa of control and experimental hamster in each group**  
 Values are expressed as mean± SD (n=6). Values that are not sharing a common superscript differ significantly at P<0.05 (DMRT).

the treatment efficacy (Manoharan *et al.*, 2004, Rajasekaran *et al.*, 2015). Extensive studies on cancer research have documented higher concentration of glycoproteins in plasma or serum and tumor tissues as well (Manoharan *et al.*, 2004, Silvan *et al.* 2011). An abnormal amount of glycoproteins was reported in oral, mammary carcinogenesis (Rajasekaran *et al.*, 2015; Manoharan *et al.*, 2011; Patel *et al.*, 1990). Plasma protein bound hexoses and hexosamines are the major constituents of plasma or serum glycoproteins of patients with cancers. Increased levels of protein bound hexose have been reported in patients with ovarian cancer (Thakkar *et al.*, 2014).

Glycoproteins levels were increased in the plasma or tumor tissues, corresponding to the tumor staging (Manoharan *et al.*, 2004). It has been reported that tumor cells synthesize increased amount of glycoproteins, which are shed into circulation during malignancy (Rajasekaran *et al.*, 2015). Increased turnover of glycoproteins in the tumor cells could thus account for increased levels of plasma glycoproteins. Several studies have pointed out that the carcinogen treatment induced higher expression of glycoproteins during abnormal cell differentiation (Sugunadevi *et al.*, 2010). It has been shown that carcinogen treated animals revealed excess amount of plasma glycoproteins, which is probably due to carcinogen induced membrane alterations during the cancerous conditions (Dabelsteen *et al.*, 1998; Suresh *et al.*, 2010). Manoharan *et al.* (2015) reported that glycoprotein levels were increased in both human and experimental oral carcinogenesis and the status of glycoproteins reflect the tumor burden and progression as well. Higher expression of glycoproteins in carcinogenesis could be due to increased activities of glycosyl transferases, sialyl transferases and fucosyl transferases (Silvan *et al.*, 2011; Rajasekaran *et al.*, 2015).

Fucose and sialic acids are observed as key biomarkers of various cancers including oral carcinoma. Fucose is one of the important sugars required for cell-cell interactions and its abnormal level play a crucial role in cancer invasion and metastasis. Sialic acid plays a pivotal role in the cancer invasion and metastasis. Profound studies documented sialic acid-rich glycoproteins in metastatic cancers (Hauselmann, 2014). Increase in sialylation has been well demonstrated in carcinogenesis (Vajaria *et al.*, 2016). Higher levels of sialic acid have been reported by several authors in tumor tissues (Bose *et al.*, 2013; Manoharan *et al.*, 2012). Patients with various forms of cancers showed higher levels of plasma total sialic acid and fucose (Chinnannavar *et al.*, 2015). The higher amount of fucose and sialic acid was reported in the tumor tissues of several cancers, including oral cancer (Kumar *et al.*, 2015, Singh *et al.*, 2012).

The observed increase in the plasma and buccal mucosa glycoproteins in hamsters treated with DMBA alone is attributed to enhanced glycoprotein turnover in the tumor cells with subsequent shedding into plasma. The present study observed reduction in the levels of plasma

and buccal mucosa glycoproteins in DMBA + *Cissus quadrangularis* leaves treated animals as compared to hamsters treated DMBA alone. The results of the present study thus reveal that the ethanolic extract of *Cissus quadrangularis* leaves has the ability to protect the cellular integrity of the buccal mucosa by preventing the glycoproteins abnormalities during DMBA induced hamsters buccal pouch carcinogenesis. The present study thus explores the modulating effect of *Cissus quadrangularis* leaves on glycoprotein status in DMBA induced oral carcinogenesis.

The protective effect of the ethanolic extract of *Cissus quadrangularis* leaves on glycoproteins abnormalities might be due to its inhibitory effect on the activities of enzymes (glycosyl transferases, sialyl transferases and fucosyl transferases) involved in the synthesis of glycoproteins. Further studies are therefore needed to analyze the activities of these enzymes in the hamsters treated with DMBA alone and DMBA + *Cissus quadrangularis* leaves treated hamsters.

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