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Research Article

PROTECTIVE EFFICACY OF *CAJANUS CAJAN* ON PLASMA AND BUCCAL MUCOSA GLYCOCONJUGATES IN DMBA INDUCED HAMSTER BUCCAL POUCH CARCINOGENESIS

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ABSTRACT

Cajanus cajan, popularly known as Pigeon pea in English, is a protein- rich medicinal plant, belongs to the family Fabaceae. It possesses a large number of secondary metabolites and some of them were reported to have diverse biological activities. Though several studies claimed multiple biological and pharmacological activities of *Cajanus cajan*, the present study has investigated the modulating efficacy of the ethonolic extract of *Cajanus cajan* leaves (CcELet) on the status of cell surface glycoconjugates (protein-bound hexose, protein bound hexosamine, sialic acid and fucose) in 7,12-dimethylbenz(a)anthracene (DMBA) induced hamster buccal pouch carcinogenesis. The tumor bearing hamster's plasma and buccal mucosa explored abnormal amount of glycoproteins as compared to control hamsters. The levels of both plasma and buccal mucosa glycoproteins were found to be declined in hamsters treated with DMBA + CcELet. The observed results thus revealed the cellular protective ability of CcELet in maintaining the cellular integrity by preventing cell surface glycoconjugates abnormalities during DMBA induced oral carcinogenesis.

INTRODUCTION

Malignant neoplasm arises due to alterations in the gene or group of genes that regulate the cell growth, structure and functions. It has been pointed out that 85% of the human cancers are due to environmental exposures (radiation, chemicals, viruses etc.). Cancerous tumors are the mass of malignant tissues, characterized by uncontrolled growth and their ability to invade or spread into local adjacent or distant tissues. Cancer cells acquire oxygen and the nutrients for their growth from the adjacent normal cells via stimulating them to form a new blood vessels, a process known as angiogenesis. The symptoms of cancers may vary depending on the cancer types and cancers are usually treated by surgery, radiation therapy and chemotherapy (Anand *et al,* 2008; Bockhorn *et al*, 2007).

Oral cancer, a cancer of the mouth or oral cavity, mostly occurs after the 4th decade of life and in general, men are affected by these forms of cancers more than twice as compared to women. It is one of the most life threatening cancers and its annual incidence is sharply increasing across the world, especially in India, Bangladesh, Sri Lanka, and Pakistan. Oral cancer usually begins in the squamous cells of the oral cavity and oral squamous cell carcinoma, thus represents 90% of all oral cancers. The persons who are habituated to tobacco smoking, chewing and alcohol use is focussed as a high risk subjects for the development of oral cancers. Though well advanced and sophisticated diagnostic and treatment modalities are available for oral cancer, the 5 year survival outcome is still poor for oral cancer patients due to late diagnosis and unawareness on oral cancer symptoms (Sankaranarayanan *et al,* 2015; Petersen, 2009).

7,12-dimethylbenz(a)anthracene (DMBA) is a widely used powerful carcinogen to induce carcinogenesis in several organs of the experimental animals, including skin, mammary and oral cancers. DMBA is used as either tumor initiator or as a complete carcinogen in cancer chemoprevention studies. The mechanism behind the carcinogenic role of DMBA includes chronic inflammation, abnormal generation of reactive oxygen species (ROS) and oxidative DNA damage. The rationale for selecting DMBA induced hamster buccal pouch carcinogenesis for the present study is due to its close similarities with human oral carcinoma at biochemical, histopathological and molecular levels (Letchoumy *et al,* 2007; Manimaran *et al,* 2016).

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Glycoproteins are complex proteins, in which the carbohydrate moiety is covalently attached to the polypeptide side chains. Glycoproteins have multiple vital functions, including cell-cell communication, cell adhesion, blood clotting and in the protection of the human body from the antigenic substances (Lipton *et al,* 1979). Sialic acids are the derivatives of neuraminic acid and N-acetyl neuraminic acid is the predominant form of sialic acid in humans. Sialic acid has been documented as a vital interactive molecule at the cell surface of the animals and exists as a terminal moiety of the sugar chain. Sialic acid contributes its vital role in several cellular interactions and recognition (Miyagi and Yamaguchi, 2010). Fucose, a hexose deoxy sugar, has been pointed out as one of the sugars needed for the optimal function of cell-cell communication. L-fucose is the predominant form and functions as an attachment site for the addition of other sugars. It has been suggested that fucose has a significant contribution in reversing the pathological diseases such as inflammation and cancer (Listinsky *et al,* 2011). The abnormal levels of sialic acid and fucose were reported in several types of cancers , including oral and mammary cancers (Patel *et al,* 1990; Chinnannavar *et al,* 2016).

Cajanus cajan, popularly known as "Pigeon pea" serves as a major protein source for the South Asian population. India is the leading country in the cultivation of Cajanus cajan and contributing around 90% of total worldwide production. The medicinal properties of this plant has been well documented in the Indian traditional system of medicines. Recent studies have explored its uses in the treatment of hepatitis, skin irritations, sores, jaundice, diabetes and cancer (Espósito Avella *et al,* 1991; Ashidi *et al,* 2010). Cajanus cajan is also used for alleviating menstrual disorders and removing bladder stones. *In vitro* and *in vivo* studies documented the tumor preventive potential of Cajanus cajan as well (Fu *et al,* 2015). The present study explores, for the first time, the protective efficacy of Cajanus cajan on cell surface glycoconjugates in DMBA induced oral carcinogenesis in golden Syrian hamsters.

MATERIALS AND METHODS

Preparation of the plant extract

Cajanus cajan leaves (500 g) were dried, finely powdered and soaked in 1500 ml of 95% ethanol overnight. The residue and filtrate were collected separately and the residue was again soaked in equal volume of 95% ethanol for further 48 h and filtered again. The two filtrates were then mixed, and the solvents were evaporated in 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

Thirty male golden Syrian hamsters with the age of 8-10 weeks old and weight of 80-100 gram were used for the present study. The hamsters were labeled into four groups of six in each. The experimental studies were carried out as per the principles and suggestions of the Institutional Ethical Committee of the Muthayammal College of the Arts and Science, Rasipuram. The experimental methodology approved and implemented for the present study is given as figure 1.

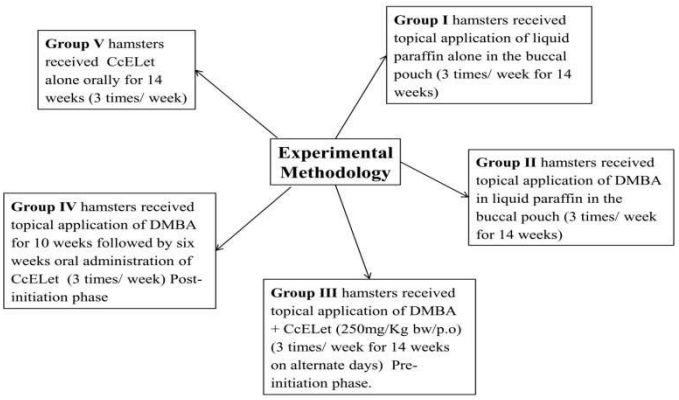

Figure 1 Experimental protocol employed for the present study

The experimental hamsters were sacrificed according to the principles of ethical committee at the end of the experimental protocol and the estimations of glycoprotiens in the plasma and buccal mucosa were carried out according to the methods mentioned in the table 1.

Table 1 Glycoproteins estimations in the plasma and buccal mucosa

Biochemical parameters	Reference
Protein bound hexose	Niebes (1972)
Protein bound hexosamine	Wagner (1979)
Total sialic acid	Warren (1959)
Fucose	Dische and Shettles (1948)

RESULTS

Figures 2 and 3 illustrate the levels of glycoproteins in the plasma and buccal mucosa tissues of the experimental hamsters respectively. We observed an accumulation of glycoproteins in both the plasma and buccal mucosa of tumor bearing hamsters (group II) as compared to control hamsters (group I). However, the levels of these glycoconjugates were found to be near

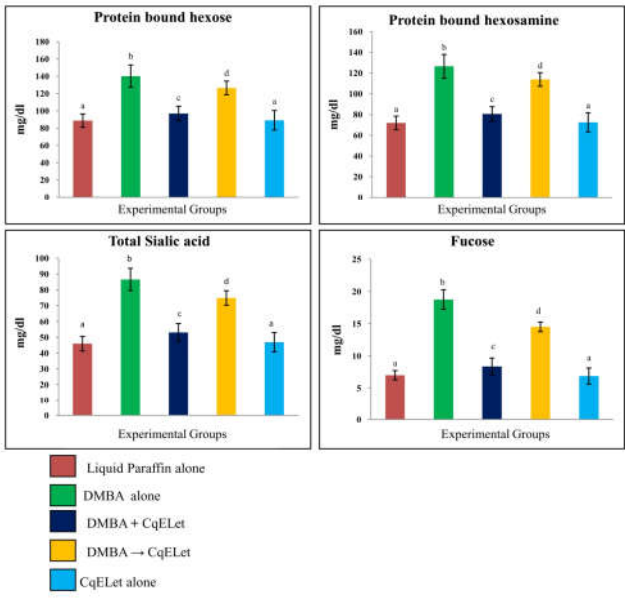

Figure 2 The levels of glycoconjugates in the plasma of control and experimental hamsters

normal range in the plasma and buccal mucosa of DMBA + CcELet treated hamsters (pre-initiation phase) and significantly decreased, in DMBA → CcELet treated hamsters (post-initiation phase). CcELet alone treated hamsters showed a glycoconjugate pattern, similar to that of control hamsters.

Values are expressed as mean± SD (n=6). Values that are not sharing a common superscript differ significantly at P<0.05 (DMRT).

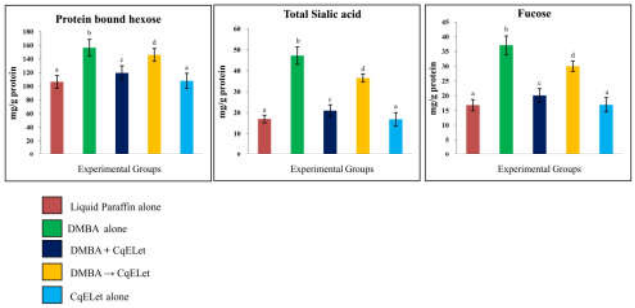

Figure 3 The levels of glycoconjugates in the buccal mucosa of Control and experimental hamster in each group.

Values are expressed as mean± SD (n=6). Values that are not sharing a common superscript differ significantly at P<0.05 (DMRT).

DISCUSSION

The effect of the ethanolic extract of the *Cajanus cajan* leaves (CcELet) was assessed on the plasma and buccal mucosa glycoconjugates status in DMBA induced oral carcinogenesis. The cell membrane components play a well-known function in malignant diseases. Glycoproteins perform their crucial biological functions in the human body in the form enzymes, hormones and blood group substances (Banerjee and Mukhopadhyay, 2016). The aberrant glycosylation pattern has been pointed out as one of the hallmark characteristics of tumor cells (Meany and Chan, 2011). Abnormal carbohydrate structures in the cell or tissues are considered as a biomarkers in various cancerous conditions, including oral cancer (Kirwan *et al,* 2015). Most of the tumor cells showed an enhanced branching of N-linked glycans and increased sialylation as well (Häuselmann and Borsig, 2014). The glycosylation pattern of cell membrane proteins and lipids altered was severely during neoplastic transformations. Extensive studies demonstrated the status of serum and tissue glycoproteins in several pathological illnesses, including carcinogenesis (Varelas *et al,* 2014; Tuccillo *et al,* 2014; Wu *et al*, 2012). Profound studies documented an elevated level of serum protein bound hexose, hexosamine, sialic acid and fucose in cancerous conditions (Evans *et al,* 1974; Bradley *et al,* 1977; Parwani and Parwani, 2011). Elevations in glycoproteins were correlated with tumor staging of cancer patients (Wolf *et al,* 1979). Wilma Delphine Silvia *et al,* (2001) have shown a gradual increase in glycoproteins in the serum of oral cancer patients with progression in tumor staging. Manoharan *et al,* (2004) showed an increase in tumor tissues glycoproteins and plasma glycoproteins in patients with oral carcinoma. Chen *et al,* (2015) reported that glycoproteins evaluation in the serum could help to assess the oral cancer invasion and metastasis. A statistical significance for the serum total sialic acid and fucose levels was demonstrated between the normal subjects and oral cancer subjects (Parwani and Parwani, 2011).

It has been suggested that the levels of glycoproteins can be utilized to correlate the tumor progression. Experimental animal studies on oral cancer clearly illustrated that the administration of carcinogen induced a typical glycosylation in the oral epithelium of tumor bearing hamsters (Dabelsteen *et al,* 1998; Manoharan *et al,* 2008). A positive correlation has been pointed out between circulatory glycoconjugates and oral cancer tumor staging (Manoharan *et al,* 2004). Ample evidence suggested an abnormal accumulation of glycoproteins in various tumor tissues which could be due to an increase in the synthesis of glycoprotein during neoplastic transformation (Kobata and Amano, 2005; Li *et al,* 2012). It has also been highlighted that the high turnover of glycoproteins in the tumor tissues could account for elevated plasma or serum glycoproteins (Song and Mechref, 2015). An increase in the glycoproteins observed in the tumor bearing hamsters (group II animals) could therefore be due to the expense of tumor tissue glycoproteins (shedding into circulation).

The levels of glycoproteins were found to be increased in patients with breast cancer and the increased was well correlated with tumor burden (Varkey *et al,* 1997). Thakkar *et al,* (2014) suggested that altered protein glycosylation pattern in ovarian cancer patients could be responsible for their higher serum glycoproteins levels. Abnormal glycoprotein levels were also demonstrated in colorectal cancer and malignant melanoma (Vedralova and Borovasnsky, 1994; Feijoo-carnero *et al,* 2004). Moreover, the increased levels of glycoproteins in the serum or plasma and in the tumor tissues were well documented in experimental cancer research, including oral and mammary carcinogenesis (Manoharan *et al,* 2008; Manoharan *et al,* 2004). Our results are in line with these previous findings and support their observations. Thus, the increased levels of glycoproteins noticed in the plasma and buccal mucosa of tumor bearing hamsters (group II) could be attributed to aberrant glycosylation pattern occurring in oral carcinogenesis.

The administration of CcELet (250mg/kg bw) to the hamsters treated with DMBA significantly reduced the levels of plasma and buccal mucosa glycoproteins in the pre-initiation phase (group III) and considerably improved the status in the post-initiation phase (group IV). The possible mechanism behind the protective efficacy of CcELet on cell surface glycoconjugates abnormalities could either be due to its inhibiting effect on the activities of enzymes involved in the glycosylation process or due to its suppressive effect on tumor formation during DMBA induced hamster buccal pouch carcinogenesis. Further studies should be needed to assess the modulating effect of CcELet on the activities of enzymes involved in the glycosylation/fucosylation/sialylation process during DMBA induced oral carcinogenesis to confirm the present research findings.

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