**INTRODUCTION**

Cancer arises due to a spectrum of pathological diseases and is characterized by rapid and abnormal cell proliferation, spreading into adjacent tissues and to distant organs. Cancer usually occurs as a result of accumulation of multiple mutations in the genetic material, DNA. The abnormal mass of tissues seen in the lips, cheeks, tongue and floor of the mouth is in general termed as oral carcinoma, the cancer of the oral cavity. Though oral cancer is life threatening, it can be treated if diagnosed early. The risk factors that are strongly linked to oral carcinogenesis include high consumption of tobacco, either in the form of smoking or chewing, and alcoholic beverages. The incidence of oral carcinoma throughout the world is steadily increasing every year, especially in developing countries, including India (Castellsagué et al., 2004; Vigneswaran and Williams, 2014).

7,12-dimethylbenz(a)anthracene is a well known chemical carcinogen and is used to develop tumors in a wide variety of animal organs, including mouth, skin and breast. This site specific carcinogen mediates cancer through inducing chronic inflammation, causing extensive DNA damage and by generating excessive reactive oxygen species (ROS). DMBA induced experimental oral carcinogenesis imitates human oral carcinogenesis at histological, molecular and biochemical aspects and thus preferred to investigate the tumor preventive potential of natural products and or synthetic constituents (Miyata et al., 1999; Manimaran et al., 2017).

Oxidative stress has been documented as one of the major risk factors in the pathogenesis of several diseases, including cancer and diabetes (Rahman et al., 2012). Oxidative stress mediated chronic inflammation has been suggested as the major mechanism in the pathogenesis of these chronic diseases. Over production of ROS in the cell can cause extensive damage to cell structure and function, which can in turn lead to neoplastic transformation. On the other hand, host cells are endowed with enzymatic and non-enzymatic antioxidants to battle against the deleterious effects of ROS. An imbalance in the role of oxidants (reactive oxygen species) and antioxidants [vitamin E, glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT)] lead to oxidative stress, which in turn cause oxidative damage to various biomolecules such as lipids, proteins and nucleic acids, there by leading to carcinogenesis (Reuter et al., 2010; Birben et al., 2012). Profound studies have also implicated the significance of phase I and II detoxification agents in carcinogenesis. Extensive
studies on carcinogenesis reported an imbalance in the activities of phase I and phase II detoxification agents (Karthikeyan et al, 2013).

*Cissus quadrangularis*, popularly known as bone setter, is used in the traditional medicine, especially to treat several feminine disorders and for joint and bone health. This plant extract has been recommended for pain, peptic ulcer, hemorrhoids, obesity, diabetes, scurvy and cancer. Extensive in vitro studies documented its cytopotential against skin cancer cell lines and Dalton’s ascitic lymphoma cell lines (Ayesha et al, 2017; Bhujade et al, 2013). *Cissus quadrangularis* explored the anti-inflammatory and gastroprotective effect in the experimental animal model as well (Netaji et al, 2015; Jainu et al, 2006). The present study, for the first time, reported the tumor preventive efficacy of *Cissus quadrangularis* leaves in DMBA induced oral carcinogenesis.

**MATERIALS AND METHODS**

**Preparation of the plant extract**

The finely powdered *Cissus quadrangularis* leaves (0.5 Kg shade dried) was soaked in 1.5 L of 95% ethanol overnight. The filtrate obtained was separated and kept in a separate flask. The left over residue was again drenched in 1.5 L of 95% ethanol for 48 h. The filtrate was collected and mixed with the previous filtrate in the flask. The solvents were evaporated with rotavapor at 40-50% under reduced pressure. The semisolid material (9%) was collected and stored at 4°C until further use.

**Experimental protocol**

The present study categorized 30 male golden Syrian hamsters (100-120g) into five groups of six each and the experiment was conducted as per the ethical guidelines of the Muthayammal College of Arts and Science, Rasiapuram, Animal Ethical Committee. The experimental protocol is given in figure 1.

All the experimental hamsters are allowed to access the pellet diet and water freely and at the end of the experimental period, hamsters were sacrificed by cervical dislocation. The plasma, liver and buccal mucosa tissues were collected from the sacrificed animals and were subjected to biochemical analysis using specific and sensitive colorimetric method (table 1).

**RESULTS**

Table 2 illustrates the tumor incidence and histopathological observations in the experimental animals. The exophytic tumor formation (well differentiated Squamous cell carcinoma) with severe hyperplasia and dysplasia was seen in all the hamsters treated with DMBA alone. The present study observed mild to moderate hyperplasia (group III) hyperkeratosis and dysplasia in the hamsters treated with DMBA + CqELet. However, no tumor formation was noticed in DMBA + CqELet treated hamsters. A significant decrease in tumor formation and tumor size with severe precancerous lesions was, however, observed in DMBA → CqELet treated hamsters (post-initiation phase).

**Table 1 Biochemical estimation carried out in the plasma and tissues**

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma TBARS</td>
<td>Yagi (1987)</td>
</tr>
<tr>
<td>Tissue TBARS</td>
<td>Ohkawa et al (1979)</td>
</tr>
<tr>
<td>Plasma vitamin E</td>
<td>Desai (1984)</td>
</tr>
<tr>
<td>Tissue vitamin E</td>
<td>Palan et al (1991)</td>
</tr>
<tr>
<td>Reduced glutathione</td>
<td>Beutler and Kelley (1963)</td>
</tr>
<tr>
<td>Oxidized glutathione</td>
<td>Tietze (1969)</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>Kakkar and Viswanathan (1984)</td>
</tr>
<tr>
<td>Catalase</td>
<td>Sinha (1972)</td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>Rotruck et al, (1972)</td>
</tr>
<tr>
<td>Glutathione reductase</td>
<td>Carlberg and Mannervik (1985)</td>
</tr>
<tr>
<td>DT-diaphorase</td>
<td>Ernster (1967)</td>
</tr>
<tr>
<td>Cytochrome P450 and b5</td>
<td>Omura and Sato (1964)</td>
</tr>
</tbody>
</table>

**Table 2 Tumor incidence and Histopathological observations of control and experimental hamsters (n=6)**

<table>
<thead>
<tr>
<th>Tumor incidence (%)</th>
<th>Control hamsters</th>
<th>DMBA treated hamsters</th>
<th>DMBA → CqELet treated hamsters</th>
<th>CqELet alone treated hamsters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of tumors</td>
<td>16 (6 animals)</td>
<td>3 (2 animals)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tumor volume (mm³)</td>
<td>305.2±28.4</td>
<td>49.5±3.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tumor burden (mm³)</td>
<td>814.2±83.6</td>
<td>75.1±6.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hyperkeratosis</td>
<td>+++</td>
<td>++ to ++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>+++</td>
<td>++ to ++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>+++</td>
<td>++ to ++</td>
<td>++ to +++</td>
<td>-</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>-</td>
<td>Observed in all hamsters</td>
<td>Observed in two hamsters</td>
<td>-</td>
</tr>
</tbody>
</table>

+ Mild, ++ Moderate, +++ Severe.

Values are represented as mean ± SD. Tumor volume was measured using the formula, v=4/3π [D1/2] [D2/2] [D3/2], where D1, D2 and D3 are three diameters of the tumors. Tumor burden was calculated by multiplying the tumor volume and the number of tumors per animal.

The activities of phase I and II detoxification agents [Cytochrome P450 (CYT P450), Cytochrome bs (CYT bs), glutathione-S-transferase (GST), Glutathione reductase (GR), DT-Diaphorase] and the status of Thiobarbituric acid reactive substances (TBARS) and antioxidants [Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), Vitamin E)] were illustrated in figures 2 to 5. The status of the above
said biochemical variables found to be significantly altered in hamsters treated with DMBA alone as compared to control hamsters. However, the status of these biomarkers was found to be reverted in DMBA + CqELet treated hamsters. A significant improvement in the status of the biochemical markers was also noticed in the DMBA → CqELet treated hamsters.

Figure 2 Buccal mucosa TBARS and antioxidants in control and experimental hamsters [n=6].

Values are expressed as mean ± standard deviation. Values that do not share a common superscript between two groups differ significantly at P<0.05 (DMRT). A - the amount of enzyme required to inhibit 50% NBT reduction; B - micromoles of hydrogen peroxide utilized/s; C - micromoles of glutathione utilized/min.

Figure 3 Plasma TBARS and antioxidants of control and experimental hamsters [n=6].

Values are expressed as mean ± standard deviation. Values that do not share a common superscript between two groups differ significantly at P<0.05 (DMRT). A - the amount of enzyme required to inhibit 50% NBT reduction; B - micromoles of hydrogen peroxide utilized/s; C - micromoles of glutathione utilized/min.

DISCUSSION
The present study makes use of the status of detoxification agents, lipid peroxidation by-products (TBARS) and antioxidants to validate the tumor inhibiting potential of CqELet in experimental oral carcinogenesis. ROS explored its harmful effect in mediating tumor promotion and progression. ROS mediated cell signaling pathways play diverse roles in all the steps of carcinogenesis, including tumor cell survival. It has been reported that lowered activities of catalase and superoxide dismutase in the tumor tissues favor tumor cell proliferation.
Phase II detoxification is involved in the deactivation of xenobiotics and drugs via phase I and phase II detoxification agents. The harmful or toxic chemical substances are metabolically activated by phase I detoxification agents into less harmful or sometimes more harmful substance, depending on the amount of exposure to the liver. During this metabolic conversion, excessive ROS are generated as well, which further complicate the oxidative damage to lipids, proteins and DNA. Phase II detoxification agents are involved in the deactivation or detoxification of xenobiotic substances. They facilitate the excretion of these substances via urine or bile by catalyzing the conjugation reaction with glutathione or glucuronic acid. Any defect in the activation of either phase I or phase II detoxification mechanism could lead to accumulation of potent carcinogetic metabolites, thereby leading to neoplastic transformation (Gu et al., 2012; Manimaran and Manoharan, 2018; Rajasekaran et al., 2015). A large number of studies pointed out defective detoxification cascade in several cancers, including oral cancer (Palanimuthu et al., 2012; Bakaran et al., 2012). Repeated and frequent topical application of the carcinoegen, DMBA, on the buccal pouch of the hamsters could account for the increased activities of phase I detoxification agents in the buccal mucosa and the liver of the hamsters treated with DMBA alone. The uneveness in the activities of phase II detoxification agents in the liver (decreased) and buccal mucosa (increased) clearly indicates the defect in the excretion of carcinogetic metabolites in the hamsters treated with DMBA alone. Oral administration of CqELet to DMBA treated hamsters maintained the status of phase I and II detoxification agents in the liver and buccal mucosa in the pre-initiation phase and significantly improved the activities in the post-initiation phase. The results of the present study thus explore the protective efficacy of CqELet in restoring the activities of detoxification agents to inhibit the formation of oral tumors in DMBA induced hamster buccal pouch carcinoegenesis.

The results of the present study conclude that CqELet inhibited the formation of tumors in the buccal pouch of hamsters treated with DMBA alone through its antilipid peroxidative ability and by improving the detoxification mechanism to effectively excrete the carcinogetic metabolites. Though CqELet inhibited the formation of oral tumors in the buccal pouch of the DMBA treated hamsters, the observed precancerous lesions such as hyperkeratosis, hyperplasia and dysplasia in the pre-initiation phase and small size tumors in the post-initiation phase warrants further extension of the duration of the experimental period to confirm, whether CqELet has the tumor inhibiting potential (chemopreventive effect) or delaying the tumor formation during DMBA induced oral carcinoegenesis.

Acknowledgement
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References


(Manoharan et al., 2005; Anbalagan et al., 2017). ROS such as superoxide radicals and hydrogen peroxides have accumulated in the tumor cells during carcinoegenesis. Excessive generation of ROS persuades DNA damage and genetic instability as well (Liu et al., 2010; Choudhari et al., 2014). Profound studies reported over production of lipid peroxidation by-products in the circulation in cancerous conditions. Studies have documented decreased levels of TBARS in various tumor tissues (Manoharan et al., 2005; Anbalagan et al., 2017). Also, studies have shown compromised lipid peroxidation and antioxidant status in the tumor tissues of and circulation of solid tumors bearing animals (Manoharan et al., 2012; Kolanjiappan et al., 2003). It has been suggested that ROS exert a synergistic role with therapeutic antioxidants in the prevention of early carcinoegenesis (Choudhari et al., 2014; Waris and Ahsan, 2006; Barrera, 2012).

In the present study, low levels of TBARS and inequity of the antioxidants was noticed in tumor tissues as compared to normal oral tissues. A large number of studies claimed low content of PUFA in the tumor tissues could be responsible for the decreased levels of TBARS. The abnormalities in the structural integrity and functions have been reported in the tumor tissues. Previous reports from our laboratory pointed out the uneveness in the antioxidant defense mechanism accompanied by a decrease in TBARS in the tumor tissues of the oral cavity (Manoharan et al., 2012; Kolanjiappan et al., 2003). The present results are in line with our previous findings. Elevated glutathione peroxidase activity and its co-substrate reduced glutathione and increase in vitamin E content in the tumor tissues are partly responsible for the reduced oxidative stress in the tumor tissues. The exhaustion of SOD and CAT activities in the tumour tissues might be due to excessive generation of superoxide radicals and hydrogen peroxide in the tumour tissues. On the other hand, insufficient antioxidant defense mechanism, as evidenced by lowered activities of SOD, CAT and GPx and diminished content of reduced glutathione and vitamin E, could be attributed to enhanced TBARS levels in the plasma. The low content of plasma vitamin E and glutathione reflects their utilization by the oral tumor tissues to favor for their abnormal cell proliferation. Hamsters treated with DMBA +CqELet revealed a significant improvement in the antioxidant defense mechanism in the pre-initiation phase, which clearly focuses the antilipid peroxidative efficacy of CqELet during DMBA induced oral carcinoegenesis. The results also imply the presence of antioxidant principles in the CqELet. A considerable improvement in the lipoperoxidation and antioxidant status was noticed in the DMBA→ CqELet treated hamsters as well.

Liver plays a putative and prominent role in the detoxification of xenobiotics and drugs via phase I and phase II detoxification agents. The harmful or toxic chemical substances are metabolically activated by phase I detoxification agents into less harmful or sometimes more harmful substance, depending on the amount of exposure to the liver. During this metabolic conversion, excessive ROS are generated as well, which further complicate the oxidative damage to lipids, proteins and DNA. Phase II detoxification agents are involved in the deactivation or detoxification of xenobiotic substances. They facilitate the


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