



## Vanillin Modulates the Status of Glycoconjugates in Favour of Tumor Suppression in 7,12dimethylbenz[a]anthracene Induced Oral Carcinoma in Golden Syrian Hamster

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### ABSTRACT

Vanillin (4-hydroxy-3-methoxybenzaldehyde), the major component of natural vanilla has been reported to show antimicrobial, anti-inflammatory, hepatoprotective and anticarcinogenic activities. In this study, the modulatory efficacy of vanillin on plasma and buccal mucosa glycoconjugates has been investigated in 7,12dimethylbenz[a]anthracene (DMBA) induced hamster buccal pouch carcinogenesis. Oral tumors were induced in the hamster's buccal pouch using 0.5% DMBA, in liquid paraffin (topical application, three times a week for 14 weeks). Plasma and buccal mucosa glycoconjugates status were assessed colorimetrically in the experimental hamsters. While the glycoconjugates status was found to be abnormal in both the plasma and buccal mucosa, vanillin administration orally at the dose of 200 mg/kg b.w to hamsters treated with DMBA reverted the glycoconjugates status to near normal concentration. The results obtained clearly exhibited the protective effect of vanillin on the cell surface and circulatory glycoconjugates in DMBA induced oral carcinoma.

**Keywords:** Oral cancer, Vanillin, DMBA, Glycoconjugates, Hamster.

### Introduction

Abnormal proliferation of cells, invasion, metastasis, and defect in apoptosis and angiogenesis are focused as key hallmark properties of cancer.<sup>1</sup> Oral carcinoma is one of the predominant malignant cancers worldwide with around 300,000 new cases per year.<sup>2</sup> While oral carcinoma accounts for lower incidences in Western countries, developing countries recorded the highest incidence, every year and accounting for about 30-40% of all carcinomas. Oral cancer arises mainly due to tobacco and betel quid chewing, tobacco smoking, and excessive consumption of alcohol.<sup>3</sup> Experimental animal models are essential to investigate the causes and pathology of diseases as well as to study the treatment of human diseases.<sup>4</sup> Animal models are also utilized to assess the stages of the malignancy.<sup>5</sup> 7,12dimethylbenz[a]anthracene (DMBA) induced tumors in the hamster buccal pouches have been widely employed to study the histopathological, genetic, and biochemical changes in oral cancer.<sup>6-8</sup> Golden Syrian hamsters act as an ideal model for oral cancer chemoprevention program as it resembles most of the characteristics of human oral cancer.<sup>9,10</sup> DMBA, a polycyclic aromatic hydrocarbon, and a potent immunosuppressor, is widely employed as a potent carcinogen to develop tumors in experimental animals.<sup>10</sup> Glycoproteins are composed of proteins and carbohydrates, which are covalently joined with each other<sup>11,12</sup>. Glycoconjugates are the vital constituents of the cell membrane and are essential for cell membrane

Functions.<sup>13</sup> Any abnormalities in the levels of glycoconjugates could lead to malignant transformation and the same has been pointed out in various cancers including oral carcinoma.<sup>11,14</sup> An increase in sialylation and fucosylation was explored as a key factor in cell transformation.<sup>15</sup>

Vanillin the major component of natural vanilla is used as a food preservative due to its anti-microbial properties.<sup>16</sup> Reports suggested that the worldwide consumption of vanilla is around 2000 tons.<sup>17,18</sup> Vanillin protected photosensitization induced oxidative stress in rat liver through its antioxidant property<sup>19</sup>. Vanillin inhibited mutagenesis induced by chemical and physical mutagens<sup>20</sup>. Vanillin has also been suggested for use in the prevention and treatment of sickle cell anemia.<sup>21</sup> Vanillin has been explored for its antioxidant, antimutagenic, and anticarcinogenic effects<sup>22-25</sup>. Vanillin suppressed several colon tumors in rat models<sup>25</sup>. Vanillin inhibited chromosomal aberrations caused by ultraviolet rays.<sup>19,24,25</sup> The present study assesses the modulating effect of Vanillin on plasma and buccal mucosa glycoconjugates against DMBA induced oral cancer in the buccal pouch of golden Syrian hamsters.

### Materials and Methods

#### Experimental animals

The present study utilized hamsters as an experimental model to develop tumors in the buccal pouch as well as to study the glycoconjugates pattern in the cell surface and circulation. The animals [male; 80-120g; 7-8 weeks old] were acquired from the Biogene, Bangalore, India and housed in the Central Animal House of Annamalai University as per the guidelines of the institutional ethics committee [Registration No. 160/1999/CPCSEA and Application No. IAEC – 1227 / 1 / 19 dated May 25.1. 2019].

#### Experimental design

It's an in vivo experimental study to explore the protective effect of vanillin, in golden Syrian hamsters which were categorized into four groups of six hamsters in each experimental group.

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Group, I experimental animals were painted 0.5% topically in the buccal pouches with liquid paraffin alone for 14 weeks (thrice a week).

Group II experimental animals were painted with 0.5% DMBA alone using No.4 paintbrush in the buccal pouches for 14 weeks (thrice a week).

Group III experimental animals received vanillin (200mg/kg b.w; orally) on alternate days of DMBA painting (thrice a week) for 14 weeks.

Group IV experimental animals received vanillin alone (200mg/kg b.w; orally) for 14 weeks (thrice a week).

#### Samples

Glycoconjugates were analyzed in the plasma and buccal mucosa colorimetrically.

#### Estimation of glycoconjugates

The plasma was treated with 95% ethanol and the residue obtained was employed for the estimation of glycoconjugates using a specific and sensitive colorimetric method. Similarly, the fresh buccal mucosa tissues excised from the experimental animals were subjected to glycoconjugates estimation after treating the tissues with chloroform and methanol. The concentration of protein-bound hexose, protein-bound hexosamine, sialic acid, and fucose in the plasma/ buccal mucosa was determined according to the procedure of Niebes<sup>26</sup>, Wagner<sup>27</sup>, Warren<sup>28</sup> and Dische<sup>29</sup> respectively. The defatted tissues are treated with 0.1N sulphuric acid and were idealized at 80°C for an hour after cooling the aliquot was used for the estimation of sialic acid. The remaining solution was treated with 0.1N NaOH and kept in an ice bath for an hour and then used for the estimation of protein-bound hexose and fucose. The protein concentration in the tissues was assayed according to the method of Lowry *et al.*<sup>30</sup>

#### Statistical analysis

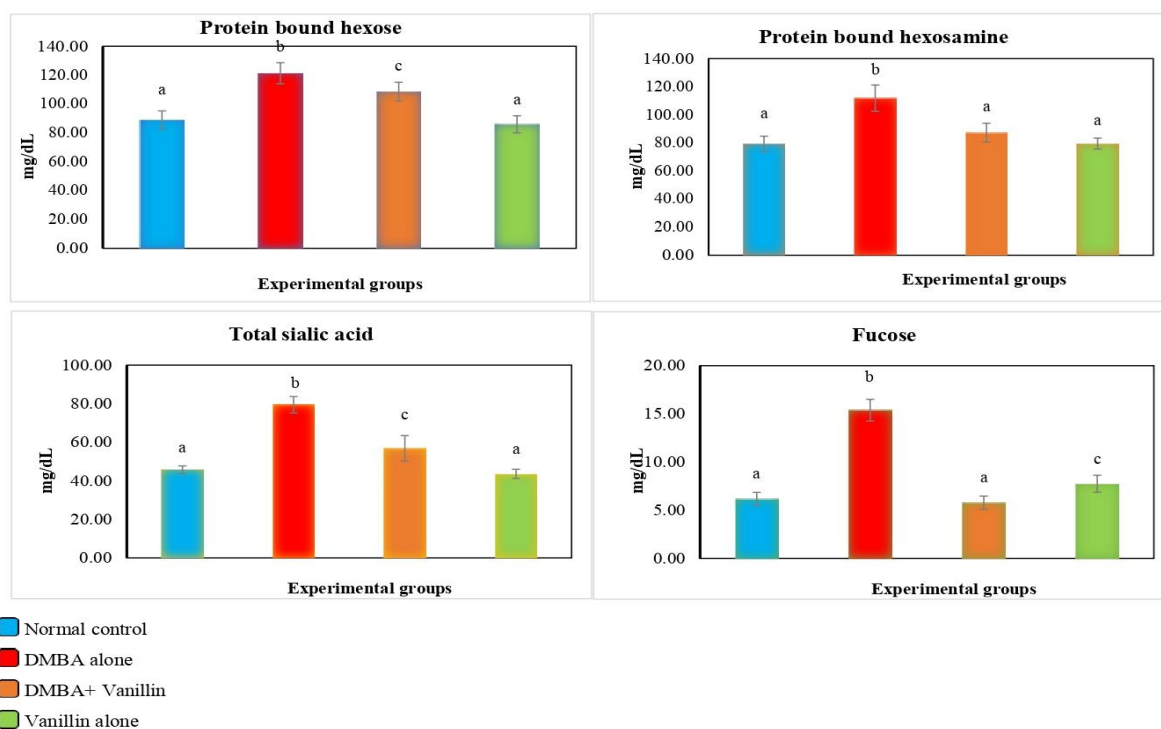
The results obtained for glycoconjugates are presented as mean  $\pm$  SD. One-way analysis of variance followed by Duncan's multiple range

tests was utilized to assess the statistical significance between the experimental groups. The p values of 0.05 or less between the two groups were considered statistically significant.

#### Results and Discussion

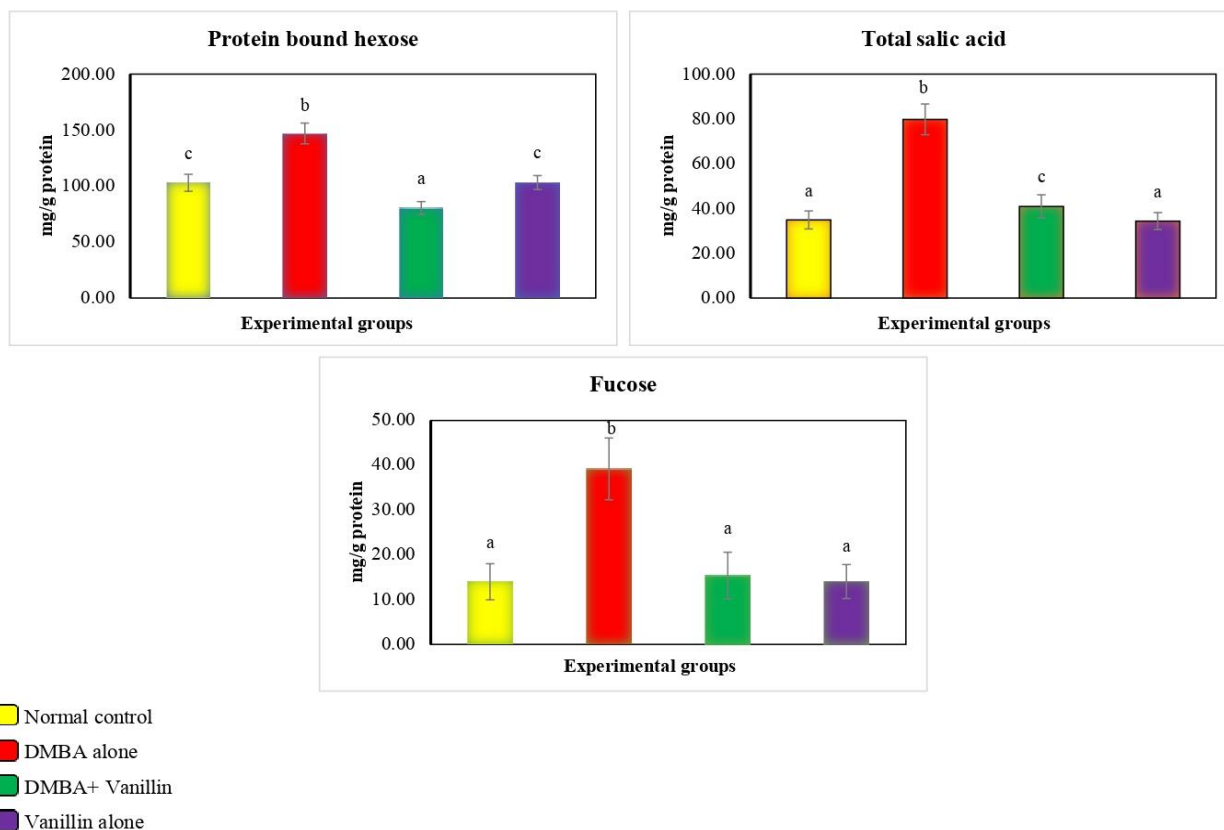
Glycoconjugates levels in the plasma and buccal mucosa are shown in Figures 1 and 2 respectively. The present study revealed elevated concentrations of protein-bound hexose, protein-bound hexosamine, sialic acid, and fucose in the hamsters treated with DMBA alone. The levels of above glycoconjugates were reduced in the hamsters treated with DMBA + Vanillin. Control hamsters and vanillin alone treated hamsters showed a similar pattern of glycoconjugates in both plasma and buccal mucosa.

Glycoproteins are covalent conjugates of proteins and carbohydrates. They play their role in several biological functions in the human body in the form of enzymes, hormones, and blood group substances.<sup>31</sup> Glycoproteins are the vital constituents of the biological plasma membrane and cell surface and any abnormalities in the level of the defect in the function of glycoproteins could lead to several pathological disorders including carcinogenesis.<sup>31,32</sup> The recent study examined the modulating effect of vanillin on plasma and buccal mucosal glycoconjugates during DMBA induced oral cancer. Glycoprotein has been utilized as a vital biomarker to diagnose the cancerous conditions as well as to assess tumor staging.<sup>33</sup> Aberrant glycosylation in the cell surface has been recognized or pointed out as a hallmark of cancers.<sup>34</sup> Profound studies pointed out an increase in the synthesis of glycoproteins in the tumor cells.<sup>35,36</sup> Tumor tissues and plasma from oral cancer patients and experimental animals bearing oral tumors showed abnormal levels of glycoproteins.<sup>37, 38</sup> Increased synthesis accompanied by a loss in epithelial cell surface carbohydrates as well as shedding into circulation has been documented well in oral cancerous conditions.<sup>39,40</sup>



**Figure 1:** Plasma glycoconjugates in control and experimental animals in each group

Values are expressed as mean  $\pm$  SD (n=6). Values that are not sharing a common superscript differ significantly at  $p < 0.05$



**Figure 2:** Buccal mucosa glycoconjugates in control and experimental animals in each group

Values are expressed as mean  $\pm$ SD (n=6). Values that are not sharing a common superscript differ significantly at  $p < 0.05$

Previous studies have also highlighted that rapidly proliferating solid tumors showed a higher concentration of glycoproteins, especially double the concentration of total sialic acid as compared with the normal cellular counterparts.<sup>41,43</sup> As a result, the current study suggests that a higher amount of glycoproteins in cancer cells and plasma of tumor-bearing hamsters (group II) could be due to abnormal synthesis of glycoproteins in the malignant tissues with subsequent shedding into the plasma. Fucose has been documented to have its vital role in tumor metastasis.<sup>37,43</sup> Fucose and sialic acid levels have been correlated with tumor staging and prognosis.<sup>41,43,44</sup> Vanillin administration (200mg/kg b.w) orally to DMBA treated hamster significantly reduced the concentration of protein-bound hexose, protein-bound hexosamine, sialic acid, and fucose in the plasma and protein-bound hexose, sialic acid, and fucose in the buccal mucosa, which implies the modulating effect of vanillin on the status of cell surface glycoconjugates towards inhibition of abnormal proliferation of cells while DMBA induced oral carcinoma. The modulating effect of vanillin could be due to its inhibitory effect on the enzyme activities that are concerned with glycoprotein synthesis. More research is thus justified to explore the impact of vanillin upon the enzyme activities concerned in the process of glycosylation, sialylation, or fucosylation process, which could help to better understand the protective efficacy of vanillin on glycoconjugates status in DMBA induced oral carcinogenesis.

## Conclusion

Vanillin has the potential to protect carcinogen-induced cell surface glycoconjugates abnormalities occurring in oral carcinogenesis of golden Syrian hamsters.

## Conflict of interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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