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RESEARCH

Anti-Tumour and Anti-Oxidative Potential of Diosgenin against 7, 12-Dimethylbenz(a)anthracene Induced Experimental Oral Carcinogenesis

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Abstract The ultimate aim of the present study was to exploring the chemopreventive efficacy of diosgenin on 7,12-dimethylbenz(a)anthracene (DMBA) induced hamster buccal pouch carcinogenesis. The chemopreventive potential of diosgenin was evaluated by measuring the tumour incidence, tumour volume and tumour burden as well as analyzing the activities of detoxification agents, levels of lipid peroxidation byproducts and antioxidants status by specific colorimetric methods. Oral squamous cell carcinoma (OSCC) was developed in the buccal pouches of male Syrian golden hamsters by painting with 0.5% DMBA in liquid paraffin, thrice a week for 16 weeks. DMBA painted animals were indicating the morphological changes as depicted as hyperplasia, dysplasia and well-developed squamous cell carcinoma. Moreover, antioxidants and lipid peroxidation byproducts levels were drastically altered in DMBA painted hamsters. Oral administration of diosgenin (80 mg/kg bw) to DMBA painted hamsters on alternate days for 16 weeks significantly reduced the formation of oral tumour and normalized the above biochemical abnormalities. We conclude that the diosgenin is probably potent chemopreventive agent due to their antioxidant function in DMBA induced hamster buccal pouch carcinogenesis.

Keywords Antioxidants · Lipid peroxidation · Diosgenin · DMBA · Histopathology

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Abbreviations

DMBA	7,12-Dimethylbenz(a)anthracene
OSCC	Oral squamous cell carcinoma
HBP	Hamster buccal pouch
PAHs	Polycyclic aromatic hydrocarbons
ROI	Reactive oxygen intermediates
RBC	Red blood cell
CAT	Catalase
SOD	Superoxide dismutase
GPx	Glutathione peroxidase
GSH	Reduced glutathione
GR	Glutathione reductase
GST	Glutathione-S- transferase
NADPH	Nicotinamide adenine dinucleotide phosphate
TBARS	Thiobarbituric acid reactive substances

Introduction

Squamous cell carcinoma (SCC) of the oral cavity is the fifth most common cancer worldwide with an incidence of 300,000 new cases annually [1]. In central to Southeast Asian countries, OSCC is the most frequent cancer and constitutes about a third of all cancers due to the practice of betel-quid chewing and or tobacco for the high incidence of oral cancer in these regions [2, 3]. The hamster cheek pouch model is the most relevant known animal system that closely related to the human oral tumours like morphogenesis, phenotype markers and genetic alterations [4]. The buccal pouch of the Syrian hamster serves an excellent target organ for chemo-intervention because of easy accessibility and follow-up of lesions. The hamster buccal pouch (HBP) mucosa is covered by a thin layer of keratinized stratified squamous epithelium

that is similar in its thickness to the floor of the mouth and the ventral surface of the tongue in humans [5].

DMBA, a potent organ and site specific carcinogen is commonly used to induce buccal pouch carcinogenesis in hamsters [6, 7]. DMBA is bioactivated by the continuous actions of cytochrome P_{450} and epoxide hydrolase to form dihydro-diol-epoxide, an elecrophilic ultimate carcinogen, establish the carcinogenic process by inducing chronic inflammation and through the over production of reactive oxygen species (ROS) [8]. Several studies were reported that evidence for the role of ROS in the pathogenesis of several cancers including oral cancer by experimentally and clinically. The quantification of phase-II detoxification enzymes such as glutathione-S-transferase (GST), glutathione reductase (GR) and reduced glutathione (GSH) in the liver may help to determine the chemopreventive potential of medicinal plants and their constituents. GSTs are a family of enzymes that play crucial role in cellular detoxification process by catalyzing the conjugation of GSH to hydrophobic electrophilic compounds (eg., organic peroxides, alkylating antineoplastic drugs) [9]. Members of this enzyme family exhibit broad and overlapping substrate specificities towards a large number of mutagenic, carcinogenic and pharmacologically active substances. GR catalyses the NADPH-dependent reduction of glutathione disulfide to GSH. Several oral cancer related studies, revealed that there is an imbalance was observed in phase II detoxification enzymes in liver [10].

During pathological conditions and oxidative stress, an imbalance in oxidant and antioxidant status has been well reported in both human and experimental studies. Reactive oxygen intermediates (ROI) including hydroxyl radical ($^{\bullet}OH$), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and nitric oxide (NO) mediated lipid peroxidation can cause oxidation events and also lead to damage the biomolecules like nucleic acids, proteins and carbohydrates [11]. Lipid peroxidation plays a vital role in the regulation of cell cycle and function. An abnormal relationship between lipid peroxidation and the rate of cell division has been observed [12]. However, the fundamental defence line of the organism against reactive oxygen species includes non-enzymatic (vit—E, C and GSH) and enzymatic (CAT, SOD, GPx) antioxidants.

Chemoprevention is a novel and promising approach to control, inhibit or suppress the tumour formation using naturally occurring phytochemicals and/or chemically synthesized derivatives. In the present study, Oral administration of diosgenin has been associated in part to their ability to scavenge free radicals and physically quench singlet molecular oxygen. It also has an anticancer effect and suppresses the growth of tumour and induces apoptosis in human mammary carcinoma [13], colon carcinoma [14], osteosarcoma [15], leukemia and erythroleukemia cells [16]. Diosgenin [(25R)-5α–spirosten-3β-ol] is a steroid sapogenin found in several plants, including Dioscorea species, Fenugreek, and Costus specious. It has over-indulged to the modification of carcinogen detoxification enzymes, such as cytochrome P_{450} enzymes [17]. Therefore the current study aimed at contributing to the potential introduction of diosgenin as a possible chemopreventive agent for hamster buccal pouch carcinogenesis.

Materials and Methods

Animals

Eight to ten weeks old male golden Syrian hamsters (*Mesocricetus auratus*) weighing 80–120 g were purchased from National Institute of Nutrition, Hyderabad, India and were maintained in Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University. The hamsters were housed in polypropylene cages and provided with standard pellet diet (Agro Corporation Private Limited, Bangalore, India) and water ad libitum. The hamsters were maintained under controlled conditions of temperature (27± 2°C) and humidity (55±5%) with a 12 h light/dark cycle.

Chemicals

7,12-dimethylbenz(a)anthracene and Diosgenin were obtained from Sigma-Aldrich Chemicals Pvt. Ltd., Bangalore, India. All other chemicals used were of analytical grade.

Experimental Protocol

The Institutional Animal Ethics Committee (Reg.No.160/1999/CPCSEA), Annamalai University, Annamalainagar, India, has approved the experimental design. A total of 40 hamsters were randomized into four groups of ten animals each. Group I animals were served as control and were painted with liquid paraffin (vehicle) thrice a week for 16 weeks on their left buccal pouches. Groups II and III animals were painted with 0.5% (No.4 Brush) DMBA in liquid paraffin thrice a week for 16 weeks on their left buccal pouches. Group II animals received no other treatment. Oral administration of diosgenin [80 mg/kg bw (diosgenin was suspended in 1% gum acacia)] to group III animals were started 1 week before the exposure of the carcinogen and continued on alternate days to DMBA painting, until the sacrifice of the animals. Group IV animals were received diosgenin alone as like group III throughout the experimental period. The experiment was terminated at the end of 16th week and all animals were sacrificed by cervical dislocation. Biochemical studies were conducted on

plasma, lysate, liver homogenate and buccal mucosa of control and experimental animals in each group.

Biochemical Estimations

Lipid peroxidation was estimated as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) by the method of Ohkawa et al. [18]. The colour formed by the reaction of thiobarbituric acid with break-down of lipid peroxidation was measured colorimetrically at 532 nm. TBARS in plasma was assayed by the method of Yagi [19]. Plasma was deproteinised with phosphotungstic acid and the precipitate was treated with thiobarbituric acid at 90°C for 1 h. The pink colour formed gives a measure of the thiobarbituric acid reactive substances, which was read at 530 nm.

Superoxide dismutase (SOD) activity was assayed by the method of Kakkar et al. [20] based on the 50% inhibition of formation of NADH-phenazine methosulphate nitro blue tetrazolium (NBT) formation. The colour developed was read at 520 nm. One unit of enzyme is taken as the amount of enzyme required to give 50% inhibition of NBT reduction. The activity of catalase (CAT) was assayed by the method of Sinha [21] based on the utilization of H_2O_2 by the enzyme. The colour developed was read at 620 nm. One unit of the enzyme is expressed as μ moles of H_2O_2 utilized per min. The activity of glutathione peroxidase (GPx) was determined using the method of Rotruck et al. [22] based on the utilization of reduced glutathione by the enzyme. One unit of the enzyme is expressed as μ moles of GSH utilized per min.

The reduced glutathione (GSH) levels in plasma, erythrocytes, liver and buccal mucosa were determined by the method of Beutler and Kelly [23]. The technique involves protein precipitation by meta-phosphoric acid and spectrophotometric assay at 412 nm of the yellow colour derivative obtained by the reaction of the supernatant with 5,5-dithiobis-2-nitrobenzoic acid.

The activity of glutathione-S- transferase (GST) in liver tissue homogenate was assayed by the method of Habig et al. [24]. GST activity was measured by incubating the tissue homogenate with the substrate 1-chloro 2,4-dinitrobenzene (CDNB). Glutathione reductase (GR) activity in liver tissue homogenate was assayed by the method of Carlberg and Mannervik [25]. The enzyme activity was assayed by measuring the formation of reduced glutathione when the oxidized glutathione (GSSH) is reduced by reduced nicotin-amide adenine dinucleotide phosphate (NADPH).

The level of plasma vitamin C was determined by the method of Omaye et al. [26]. The dehydro-ascorbic acid formed when the oxidation of vitamin C by copper, forms a coloured product on treatment with 2,4-dinitrophenylhy-drazine, whose absorbance was measured at 520 nm. Vitamin E levels in plasma and erythrocyte membrane were determined colorimetrically by the method of Desai [27]. Vitamin E presents in the lipid residue forms a pink coloured complex with bathophenanthroline-phosphoric acid reagent, which was measured at 536 nm. Tissue vitamin E was measured by the fluorimetric method of Palan et al. [28]. The lipid extracts were dried under nitrogen and the residue were suspended in 66% ethanol, followed by the addition of 4 ml of hexane and 0.6 ml of 60% H_2SO_4. The fluorescence intensity of vitamin E extracted to the hexane layer was measured at an excitation of 295 nm and emission of 320 nm.

Macroscopic Observation

The total number of tumour in the buccal pouches of hamsters was counted and the diameter of the each and every tumour was measured by using vernier caliper. Tumour volume was calculated using the formula, $V(4/3)\pi[(D1/2)(D2/2)(D3/2)]$ where D1,D2, and D3 are the three diameters (mm) of the tumour. Tumour burden was calculated by multiplying tumour volume and the number of tumours/hamster.

Histological Studies

Buccal mucosa was fixed in 10% buffered neutral formalin solution and routinely processed and embedded with paraffin, 2–3 μm sections were cut in a rotary microtome, picked up on clean glass slides, dried at 37°C and used for histological studies.

Statistical Analysis

The data are expressed as the mean ± SD. Statistical comparisons were performed by One-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). The results were considered statistically significant if the p values were 0.05 or less.

Results

Clinical Appearance of Oral Neoplasm

Table 1 Summarizes the incidence, volume and burden of tumour occurred in the control and experimental animals. We observed 100% tumour formation with a mean tumour volume in DMBA alone painted hamsters (Group II). Oral administration of diosgenin at a dosage of 80 mg/kg bw for 16 weeks, highly reduced the tumour incidence in DMBA painted hamsters (Group III). No tumours were observed in control animals painted with liquid paraffin alone (Group I) as well as diosgenin administered animals. (Group IV)

Table 1 Incidence of oral neoplasm in control and experimental animals in each group (n=10)

Parameters	Group I (Control)	Group II (DMBA)	Group III (DMBA + Diosgenin)	Group IV (Diosgenin alone)
Tumor incidence (Squamous cell carcinoma)	0	100%	0	0
Total number of tumors/animals	0	39(10)	3(2)	0
Tumor volume (mm^3)/animals	0^a	381.6 ± 33.9^b	29.3 ± 3.3^c	0^a
Tumor burden (mm^3)/animals	0^a	1352.4 ± 104.2^b	60.3 ± 7.2^c	0^a

Tumor volume was measured using the formula $v = 4/3\pi[D_1/2][D_2/2][D_3/2]$ where D_1, D_2, and D_3 are the three diameters (mm) of the tumor. Tumor burden was calculated by multiplying tumor volume and the number of tumor/animals. () indicates total number of animals bearing tumors

Histopathological Evaluation

Figure 1 & Table 2 Shows the histopathological features were observed in buccal mucosa of control and experimental animals. The buccal pouches from DMBA only treated hamsters revealed severe hyperkeratosis, hyperplasia, dysplasia along with well-differentiated squamous cell carcinoma were noticed (Group II). Diosgenin treatment at a dosage of 80 mg/kg bw significantly reduced those pathological changes mild to moderate preneoplastic lesions [hyperplasia, keratosis and dysplasia] were observed in Group III hamsters.

TBARS and Antioxidant Enzymes Levels in Plasma and Erythrocytes

Table 3 Shows the levels of TBARS, enzymatic and non-enzymatic antioxidants (SOD, CAT, GPx, GSH, vitamin C and E) in plasma and erythrocytes of control and experimental animals in each group, the concentration of TBARS was significantly increased whereas the statuses of antioxidants were significantly decreased in tumour bearing hamsters (Group II) as compared to control hamsters. Oral administration of diosgenin to DMBA painted hamsters (Group III) significantly revert back those alterations to the near normal concentration of TBARS and antioxidants. Hamsters treated with diosgenin alone (Group IV) showed no significant differences in TBARS and antioxidants status as compared to control animals.

TBARS, Antioxidant Enzymes Levels and the Status of Detoxifying Agents in Buccal Mucosa and Liver

Table 4 Shows the status of TBARS and antioxidants levels in the buccal mucosa of control and experimental animals

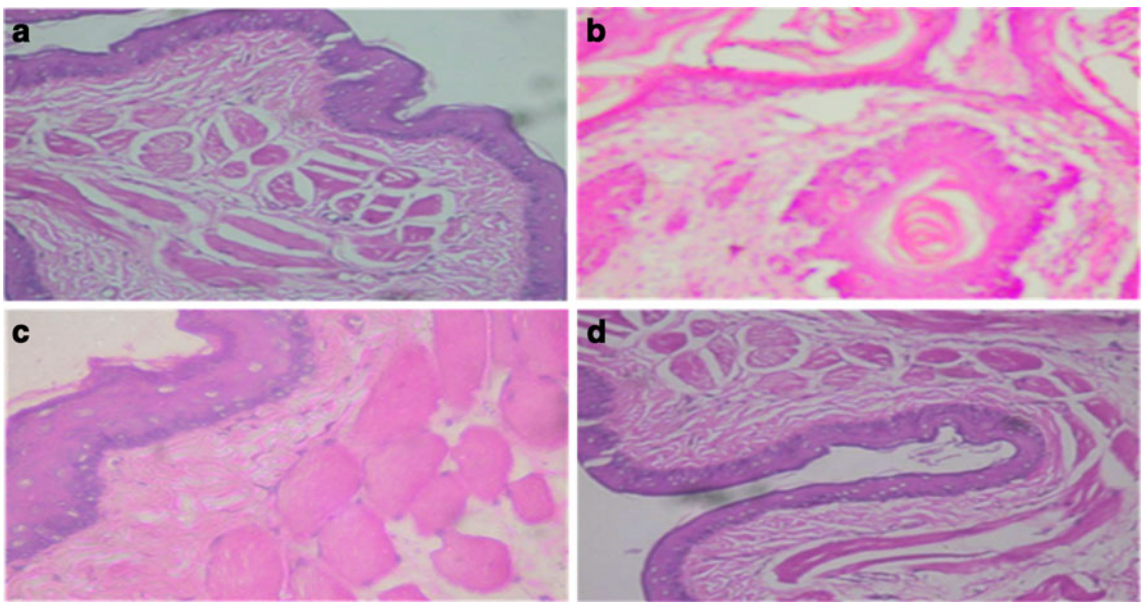

Fig. 1 Representative histopathological changes of hamster buccal mucosa. **a** Normal buccal epithelium showing control hamster, **b** DMBA alone treated hamster buccal mucosa showing hyperkeratosis, hyperplasia, dysplasia and well-differentiated squamous cell carcinoma obseved with keratin pearls, **c** DMBA + Diosgenin treated hamster showing focal mild epithelial dysplasia, and **d** Diosgenin alone treated hamster showing normal epithelium (hematoxylin/eosin stain, original magnification. 20x)

Table 2 Histopathological changes in the buccal mucosa of control and experimental animals in each group (n=10)

Groups	Hyperkeratosis	Hyperplasia	Dysplasia	Squamous cell carcinoma
Group I (Control)	Not observed	Not observed	Not observed	Not observed
Group II (DMBA)	Severe	Severe	Severe	Well differentiated
Group III (DMBA + Diosgenin)	Moderate	Moderate	Mild	Not observed
Group IV (Diosgenin alone)	Not observed	Not observed	Not observed	Not observed

in each group. Decrease in TBARS levels and disturbances in antioxidants status (SOD and CAT were decreased; GSH, GPx, and vitamin E increased) were noticed in tumour bearing hamsters (Group II) as compared to control hamsters(Group I). Oral administration of diosgenin to DMBA painted animals (Group III) restored the concentration of TBARS and antioxidants to near normal range. Hamsters treated with diosgenin alone (Group IV) showed no significant differences in TBARS and antioxidants status as compared to control animals (Group I). Consequently that Table 4 also shows the status of detoxification agents were significantly decreased in tumour bearing animals (Group II) as compared to control animals. Oral administration of diosgenin to DMBA painted animals (Group III) significantly brought back the status to near normal concentration of the enzymes. Animals treated with diosgenin alone (Group IV) showed no significant differences in the status of detoxification agents as compared to control animals.

Discussion

Oral squamous cell carcinoma is one of the most frequent neoplasm in Indian population and major public health problem in many countries including Taiwan, Bangladesh and Pakistan [29]. DMBA induced hamster buccal pouch carcinogenesis is commonly used to assess the chemopreventive potential of phytochemicals because the oral carcinoma induced by DMBA closely related to the mammalian system which is both histologically and morphophysiologically [30, 31]. We counted 100% tumour formation in the buccal pouches of hamsters treated with DMBA alone. Histopathologist confirmed the tumour was moderately differentiated squamous cell carcinoma. Oral administration of diosgenin (80 mg/kg bw) to DMBA treated hamsters significantly reduced the time taken for oral neoplasm formation or oral tumourigenesis. Therefore, Our results found that diosgenin has significant chemopreventing capacity during DMBA induced buccal pouch carcinogenesis.

Table 3 TBARS and antioxidants levels in plasma and erythrocytes of control and experimental animals in each group

Parameters	Group I (control)	Group II (DMBA)	Group III (DMBA + Diosgenin)	Group IV (Diosgenin alone)
PLASMA TBARS (nmol/ml)	2.05 ± 0.21^{a}	4.69 ± 0.34^{b}	2.31 ± 0.19^{c}	2.03 ± 0.20^{a}
GSH (mg/dl)	27.4 ± 1.9^{a}	18.3 ± 1.5^{b}	25.2 ± 2.0^{c}	27.6 ± 1.8^{a}
Vitamin-C (mg/dl)	1.35 ± 0.06^{a}	0.79 ± 0.04^{b}	1.21 ± 0.07^{c}	1.36 ± 0.08^{a}
Vitamin-E (mg/dl)	1.38 ± 0.09^{a}	0.73 ± 0.08^{b}	1.19 ± 0.11^{c}	1.39 ± 0.07^{a}
SOD (U^{A}/ml)	2.63 ± 0.18^{a}	1.70 ± 0.13^{b}	2.31 ± 0.21^{c}	2.64 ± 0.19^{a}
CAT (U^{B}/ml)	0.92 ± 0.09^{a}	0.56 ± 0.04^{b}	0.79 ± 0.08^{c}	0.93 ± 0.07^{a}
GPx (U^{C}/l)	108.7 ± 9.2^{a}	76.9 ± 7.3^{b}	93.8 ± 8.7^{c}	109.6 ± 9.1^{a}
ERYTHROCYTES TBARS (pmoles/mg Hb)	1.49 ± 0.11^{a}	2.78 ± 0.32^{b}	1.64 ± 0.13^{c}	1.48 ± 0.12^{a}
Membrane TBARS (nmol/mg protein)	0.31 ± 0.02^{a}	1.14 ± 0.09^{b}	0.46 ± 0.03^{c}	0.30 ± 0.01^{a}
Vitamin-E (μg/mg protein)	2.57 ± 0.19^{a}	1.81 ± 0.21^{b}	2.34 ± 0.18^{c}	2.58 ± 0.20^{a}
GSH (mg/dl)	51.2 ± 3.8^{a}	33.1 ± 2.6^{b}	46.3 ± 3.4^{c}	51.7 ± 3.9^{a}
SOD (U^{A}/mg Hb)	2.52 ± 0.19^{a}	1.54 ± 0.24^{b}	2.36 ± 0.20^{c}	2.53 ± 0.21^{a}
CAT (U^{B}/mg Hb)	1.37 ± 0.09^{a}	0.68 ± 0.12^{b}	1.22 ± 0.10^{c}	1.38 ± 0.11^{a}
GPx (U^{C}/g Hb)	15.59 ± 1.34^{a}	9.22 ± 0.96^{b}	13.84 ± 1.29^{c}	15.67 ± 1.31^{a}

Values are expressed as the mean ± SD for 10 hamsters in each group. Values that are not sharing a common superscript letter in the same column differ significantly at $p<0.05$(DMRT)

A—The amount of enzyme required to inhibit 50% NBT reduction; B—Micromoles of H_2O_2 utilized/s; C—Micromoles of glutathione utilized/min

Table 4 Buccal Mucosa TBARS, antioxidants status and activities of detoxification agents in liver homogenate of control and experimental animals in each group

Parameters	Group I (Control)	Group II (DMBA)	Group III (DMBA + Diosgenin)	Group IV (Diosgenin alone)
BUCCAL MUCOSA TBARS (nmol/100 mg protein)	68.7±5.9[a]	32.4±3.8[b]	59.8±4.2[c]	69.3±5.7[a]
GSH (mg/100 mg tissues)	6.03±0.51[a]	13.21±0.94[b]	7.05±0.61[c]	6.01±0.49[a]
Vitamin-E (mg/100 mg tissues)	1.66±0.18[a]	2.84±0.35[b]	1.79±0.22[c]	1.65±0.19[a]
SOD (U^A/mg protein)	4.93±0.31[a]	2.61±0.19[b]	4.78±0.28[c]	4.95±0.30[a]
CAT (U^B/mg protein)	35.7±3.2[a]	21.4±2.4[b]	32.9±3.0[c]	36.8±3.3[a]
GPx (U^C/g protein)	6.54±0.48[a]	13.1±0.79[b]	7.83±0.58[c]	6.51±0.49[a]
LIVER GSH (nmol/g tissue)	2.61±0.20[a]	1.46±0.09[b]	2.39±0.17[c]	2.63±0.21[a]
GST (nmol/CDNB conjugate formed/min/mg protein)	172.6±12.4[a]	119.3±9.3[b]	162.4±11.5[c]	173.5±12.7[a]
GR (nmol of NADPH oxidized per min/mg protein)	40.8±3.5[a]	21.3±2.1[b]	35.2±2.9[c]	41.1±3.6[a]

Values are expressed as the mean ± SD for 10 hamsters in each group. Values that are not sharing a common superscript letter in the same column differ significantly at $p<0.05$(DMRT)

A—The amount of enzyme required to inhibit 50% NBT reduction; B—Micromoles of H_2O_2 utilized/s; C—Micromoles of glutathione utilized/min

Previous data reported that chemopreventive agents catalyses the DNA damaging entities into exertable metabolites through the induction of detoxification agents [32]. Recent studies were demonstrated that any phytochemical that induces the activities of GST, GR and increased formation of GSH has significant chemopreventive potential during carcinogenesis [33]. In addition, reduces the activities of above phase-II detoxification agents were reported in several types of experimental oral carcinogenesis [34]. Oral treatment of diosgenin to DMBA induced hamsters recovered the status of phase II detoxification agents to near normal range. Moreover, Our results indicates that diosgenin enhanced the process of conjugation and modulation of carcinogen metabolizing enzymes and subsequent elimination of carcinogenic metabolites during DMBA induced oral carcinogenesis.

ROI induced oxidative stress has been evidenced in the pathogenesis of several malignancies including oral neoplasm [35]. Quantification of the plasma TBARS is considered to be a true biomarker to assess the extent of tissue damage during pathological conditions [36]. DMBA, toxic and reliable which is a known polycyclic aromatic hydrocarbon [37, 38]. Which can cause damage to biomolecules and inflammatory response upon cell damage, there by evidence of carcinogenesis [39]. Reaction of free radicals with cellular membranes may lead to the formation of lipid hydroperoxides, which have been extensively investigated as in vivo indicators of lipid peroxidation byproducts both in human and animals [40]. Thus, it must be considered a significant endogenous source of DNA damage and mutations that contribute to human genetic diseases [41, 42]. During pathological conditions, RBC and RBC membrane are disturbed by oxidative stress [43]. Other investigators have also reported that elevated levels of TBARS were observed in the plasma of tumour bearing animals [44]. Elevated levels of TBARS in plasma could therefore be due to moreover production and leakage from damaged erythrocytes and other corresponding host tissues [45]. Oral administration of diosgenin significantly decreased the levels of plasma and erythrocytes TBARS in DMBA treated hamsters, which suggest that diosgenin has antioxidant and antilipidperoxidative potential during oral carcinogenesis.

Enzymatic and non-enzymatic antioxidants are sole adventure roles against ROS mediated oxidative stress [46]. Alterations the levels of non- enzymatic antioxidants are the contributors for several types of carcinogenesis [47]. Ascorbate and α-tocopherol are chain-breaking antioxidants and have been shown to prevent lipid peroxidation and block the initiation of free radical mediated chain reaction [48]. Significantly decreased the levels of vitamins C, E and GSH content in plasma and erythrocytes are probably due to their utilization by tumour tissues to meet their demands of growing tumours in the circulation [49, 50]. Reduced the activities of SOD, CAT and GPx could be explained by a less effective protection system, in which reported that considerable decline in the fast growing tumours appear to be unique to neoplastic transformation and elevated levels of lipid peroxidation by products in circulation [51]. In the meanwhile, the inhibitory effect of diosgenin make a significant turn on HBP carcinogenesis was associated with modulation of carcinogen-metabolizing enzymes. Which is lend credibility to these observations.

Oral administration of diosgenin significantly improved the status of enzymatic and non enzymatic antioxidants in

DMBA treated hamsters which suggest antioxidant potential and free radical scavenging property during oral carcinogenesis. Although the exact mechanism of anti-tumour potential of diosgenin is not clear, its free radical scavenging property and induction of phase-II detoxification agents during DMBA induced oral carcinogenesis could play a role.

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