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Anticancer Potential of Diphenyl Carbonate-Crosslinked Cyclodextrin Nanocarrier Loaded with Allyl Methyl Sulfide in a DMBA-Induced Oral Squamous Cell Carcinoma Model

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Abstract

Oral Squamous Cell Carcinoma (OSCC) is the most common malignant tumour and the most resistant to treatment. The lack of a therapy that significantly alters its course necessitates for urgent need to create novel drug delivery formulations. By increasing accessibility and stability, lowering effective dosages and minimising therapeutic adverse effects, nanomedicine can significantly enhance the suppression of cancer cells. This study investigates the chemopreventive effects of Allyl Methyl Sulfide-Loaded Nanosponge (AMS-NS) and Allyl Methyl Sulfide (AMS) on DMBA-induced Hamster Buccal Pouch Carcinogenesis (HBPCs). By topically applying 0.5% DMBA thrice weekly for ten weeks, the left buccal pouches of male golden Syrian hamsters developed oral tumours. In our study, DMBA-treated hamsters showed 100% tumour development, changed levels of antioxidants, detoxifying agents, and Lipid Peroxidation (LPO) products as compared to control hamsters. The results of the current biochemical and histological research show that AMS-NS-treated hamsters have a tumour-reversing effect.

Major Findings: This study found that AMS-NS modified metabolic profiles (antioxidants, detoxification, and LPO) and reduced tumour formation in DMBA-induced oral carcinogenesis.

Keywords: Allyl Methyl Sulfide, Nanomedicine, Nanosponge, Novel Drug Delivery, Oral Squamous Cell Carcinoma

1. Introduction

Cancer is the second most significant cause of death globally, and its multistep processes may be broadly divided into three stages: Initiation, promotion and progression[1]. Oral cancer can affect the head and neck, tongue, buccal mucosa, oral cavity, the floor of the mouth, and the gingiva, including the lips[2]. Due to high risk factors unique to the area, including genetic, epigenetic, environmental, and behavioural influences, like alcohol and tobacco use, are the primary etiologic risk factors for 90% of oral cancer[3]. The predicted number of cancer incidents in India for

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the year 2022 is 14,61,427. The number of cancer cases is expected to grow by 12.8% in 2025 compared to 2020. Oral cancer causes around 377,000 new cases and 177,000 deaths worldwide each year[4]. The development and treatment of oral cancer by chemopreventive drugs are studied in hamsters because they have an anatomical feature resembling that of human[5].

A profound study shows that the effect of polycyclic aromatic hydrocarbon 7,12-dimethylbenz(a)anthracene (DMBA) is widely used to initiate buccal pouch cancer. It is an organ-specific procarcinogen and dihydrodiol epoxide, a reactive metabolite produced by DMBA metabolism, that binds to DNA's adenine and guanine residues and induces chronic inflammation by producing Reactive Oxidative Species (ROS), promoting mutagenesis and carcinogenesis[6].

Oxidative stress is characterised as an imbalance between ROS concentrations and antioxidant defence systems. ROS, including hydrogen peroxide, superoxide, and hydroxyl radicals, are critical in causing cell damage and loss of function in many tissues and organs. Hydrogen peroxide (H_2O_2) is a highly reactive chemical that causes cell damage. The H_2O_2-induced oxidant model has been frequently used to investigate how cells respond to oxidative stress[7].

DMBA-induced hamster buccal pouch carcinogenesis by the analysis of lipid peroxidation by-products Lipid Hydroperoxide (LOOH), Thiobarbituric Acid Reactive Substances (TBARS), and Conjugated Dienes (CD), Enzymatic Antioxidant Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPX) non-enzymatic antioxidant (Vitamin E and GSH), detoxification phase I cytochrome p450 (Cyt p450), cytochrome b5 (Cyt b5), phase II DT-Diaphorase (DTD), reduced Glutathione (GSH), Glutathione Reductase (GR), Glutathione-S-Transferase (GST) and Glutathione Disulfide (GSSG)[8]. Due to their high tolerance and lower toxicity, plant-derived chemopreventive medicines are becoming more predictable in clinical studies[9]. Chemopreventive drugs are effective in delaying the development and progression of tumours at the biochemical and molecular levels[10].

Organosulfur Compounds (OSCs) have been shown to exert various biological effects, including antioxidant and anti-inflammatory efficacy[11]. Organosulfur compounds have been demonstrated by Amagase *et al.*, to have therapeutic benefits against diabetes, cancer, cardiovascular, and liver illnesses[12]. The primary bioactive components responsible for the beneficial advantages of garlic are organosulfur compounds[13,14]. One of the main bioactive components of garlic has been discovered to be AMS. According to Wargovich *et al.*, AMS, a prominent constituent of volatile garlic metabolites, has antibacterial, antioxidant, and anticancer effects[14].

Previous studies have revealed the physicochemical characterisation and *in vitro* effects of AMS loaded onto beta cyclodextrin nanosponges. Findings demonstrated that AMS-NS reduced cancer cell development *in vitro* and to a greater extent than free AMS by cross-linking cyclodextrins, which are cyclic oligosaccharides made up of many a-D-glucopyranose units connected by an α-1,4 bond and include α-, β-, and γ-cyclodextrins carrying six and seven, or eight glucopyranose units, respectively. New biocompatible polymer nanoparticles known as cyclodextrin nanosponges can be created. Due to its strong potential to encapsulate pharmaceutical activity, beta-cyclodextrin is most frequently utilised to create nanosponges[15,16]. The crosslinker diphenyl carbonate was used to develop nanosponges containing medications[17]. By modifying the crosslinker type and quantity, the nanosponges may be customised for the chosen drug molecule to provide the desired pharmacological profile[18].

OSCs have two main mechanisms for their cellular protective effects: anti-inflammation and anti-oxidation. Bioactive compounds found in garlic have strong anticarcinogenic effects through a variety of mechanisms, such as apoptosis, cell cycle arrest, and angiogenic cascade activation. Many genes have been identified as being involved in the control of significant signalling pathways, which include JNK, Akt/Pi3K, p38, MAPK, EMT, Wnt, p53, ERK1/2, NF-κB, Nrf2, STAT3, and Chk1/cyclin B1[19,20]. This study investigates the formulation of AMS-NS as a potential chemopreventive strategy for the treatment and prevention of oral carcinogenesis. In light of this, an effort has been made in the current work to create cyclodextrin NS and AMS in conjunction as a cutting-edge, prospective delivery method as an experimental model for treating oral cancer.

2. Methods and Materials

2.1 Chemicals

AMS, DMBA, Nicotinamide Adenine Di-nucleotide Phosphate (NADPH) and GSH were obtained from Sigma-Aldrich. Beta cyclodextrin, Diphenyl carbonate,

Thiobarbituric acid, phenazine methosulfate, nitro blue tetrazolium, 2,4-dinitrophenylhydrazine, 5,5′-dithiobis (2-nitrobenzoic acid), and 1-chloro-2,4-dinitrobenzene were purchased from HiMedia Laboratories. All other chemicals were of analytical grade.

2.2 Animal

The local institutional animal ethics committee (160/1999/CPCSEA) of Annamalai University approved our experimental design (Proposal no: 1312, dated: 27.12.2021). The hamsters were maintained following the principles and guidelines of the ethical committee for animal care of Annamalai University, following the Indian National Law on animal care and use. 36 hamsters, 8-10 week-old, 80-120g male golden Syrian hamsters (Mesocricetus auratus) were used. These were purchased from Biogen, Bangalore, India and maintained in Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University. The hamsters were housed in six groups in polypropylene cages and provided a standard pellet diet and water ad libitum. The hamsters were maintained at 27 ± 2ºC and controlled humidity with a 12h light:12 h dark cycle.

2.3 DMBA-Induced Hamster Buccal Pouch Carcinogenesis

Tumours were induced in the left buccal pouches by topical application of 0.5% DMBA in liquid paraffin three times/week for ten weeks using a number 4 paint brush[21].

2.4 AMS and AMS-NS Preparation

AMS was dissolved in corn oil, AMS-NS was dissolved in water, and 1ml of the oral suspension was administered via the intragastric tube.

2.5 Experimental Design

Thirty-six male golden Syrian hamsters were divided into six groups (n=6). For ten weeks, the left buccal pouches of the hamsters in groups 1 to 5 were painted with 0.5% DMBA. No additional treatment was given for group 1 hamsters. Additionally, AMS and AMS-NS were given orally to the hamsters in groups 2 (AMS 100mg/kg b.w.), 3 (AMS-NS 25 mg/kg b.w.), 4 (AMS-NS 50 mg/kg b.w.) and 5 (AMS-NS 100 mg/kg b.w.) three times per week via an intragastric tube beginning one week before exposure to the carcinogen and continued on days alternating with the DMBA painting until the end of the experimental period. Group 6 hamsters were used as an untreated control. After an overnight fast, all of the animals were anaesthetised with ketamine chloride (60mg/kg b.w.) and sacrificed by cervical dislocation to conclude the experiment at the end of the 14th week. The number of tumours in each oral, buccal pouch was counted, and the diameter of each tumour was measured using a vernier calliper. The tumour burden has been determined by multiplying the tumour volume and the number of tumours per hamster. The tumour volume was calculated using the formula $V = 4/3\ \pi\ (D_1/2), (D_2/2)$, and $(D_3/2)$, where D_1, D_2, and D_3 represented the tumour's three diameters (mm^3). The histopathological characteristics were examined using buccal tissue from the control and experimental hamster groups and biochemical markers utilising plasma, liver, and oral mucosa tissue.

2.6 Histological Study

Buccal tissues were preserved in a 10% formalin solution, then dried in 50 to 100% ethanol before being covered in paraffin. The 2-3µm slices were cut, collected, and dried on glass slides at 37°C on a revolving microtome. It had

Table 1. Experimental design

Groups	Particular	Treatment
1	DMBA alone	0.5% DMBA for 10 weeks, three times a week
2	DMBA+AMS	AMS 100mg/kg b.w. was administered orally three times per week for 14 weeks, alternated with DMBA painting.
3	DMBA+AMS-NS	AMS-NS 25mg/kg b.w. was administered orally three times per week for 14 weeks, alternated with DMBA painting
4	DMBA+AMS-NS	AMS-NS 50mg/kg b.w. was administered orally three times per week for 14 weeks, alternated with DMBA painting
5	DMBA+AMS-NS	AMS-NS 100mg/kg b.w was administered orally three times per week for 14 weeks, alternated with DMBA painting
6	Control	No treatment

hematoxylin and eosin on it. These slides were examined with a microscope for histological pictures of OSCC. The Ribeiro *et al.*, approach created the histopathology slides[22].

2.7 Collection of Samples and Biochemical Estimation

Plasma separation required the collection of blood samples, which were subsequently centrifuged at 1000g for 15 minutes. The hamster tissue samples were homogenised in a homogeniser with a Teflon pestle using the appropriate buffer after being rinsed with ice-cold saline. Enzymatic antioxidant activity in the plasma and buccal mucosal tissue, including SOD, CAT and GPX, three enzymatic antioxidants, was found in the plasma and buccal tissues[23,24]. Vitamin E and GSH were shown to be the non-enzymatic antioxidants that lowered GSH[25]. Plasma and buccal tissues were examined for LPO by-products such as LOOH, TBARS and CD [26,27]. The techniques (Omura *et al.*, 1964; Ernsrea *et al.*, 1967) were used to measure the detoxifying enzymes such as Cyt p450, Cyt b5 and DTD, reduced GSH, GST, GR and GSSG in liver and buccal tissue, respectively[28,29]. Using the approach of Lowry *et al.*, proteins were determined[30].

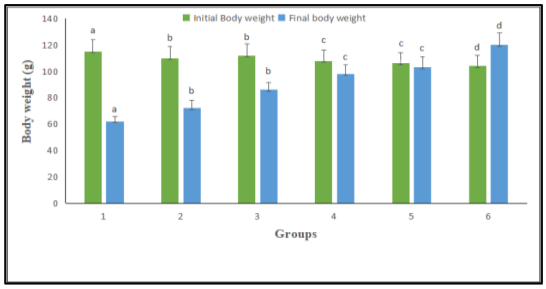


Figure 1. Body weights of control and experimental groups. Values are presented as the mean standard deviation (SD) for each set of 6 hamsters. At (a-e) $p<0.05$.

2.8 Statistical Analysis

Values are expressed as means ± SD. Comparisons were performed using One-way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) using SPSS version 14.0 for Windows (SPSS Inc., Chicago, IL). Statistical significance was set at $p<0.05$.

3. Results

3.1 Measurement of Body Weight

Figure 1 displays the difference in body weight for each group between the start and end of treatment. The hamsters treated with DMBA alone showed a significant ($p<0.05$) quit in body weight at the end of the experimental period when compared to group 6. When DMBA-painted hamsters were given AMS-NS orally at several dosages of 25, 50 and 100 mg/kg b.w., their body weight significantly improved ($p<0.05$). The body weight of group 2 (AMS 100 mg/kg b.w.) hamsters was significantly ($p<0.05$) higher than that of group 1 hamsters.

3.2 Tumour Incidence, Volume and Burden

For all groups, incidence, volume, and burden of tumours are shown in Table 1. Hamsters in group 1 who received DMBA treatment alone developed tumours at a rate of 100%, with a mean tumour volume of 275.15mm^3 and a tumour burden of 1650.9mm^3. DMBA-treated hamsters in group 2 received AMS, 3 to 5 received oral administration of AMS-NS a week before carcinogen application, at different dosages for 14 weeks, preventing the formation of OSCC compared to the control groups.

Table 2. Tumour incidence, number, volume and burden in control and experimental groups

	DMBA	DMBA+AMS (100 mg/kg b.w.)	DMBA+AMS-NS (25 mg/kg b.w.)	DMBA+AMS-NS (50 mg/kg b.w.)	DMBA+AMS-NS (100 mg/kg b.w.)	Control
Tumour incidence	100%	11.18%	8.23%	0	0	0
Total number of tumours/hamster	6 ± 4.04	1.65 ± 0.68	1.02 ± 0.28	0	0	0
Tumour volume (mm^3)	275.15 ± 18.18	22.25 ± 1.36	20.12 ± 1.12	0	0	0
Tumour burden (mm^3)	1650.9 ± 66.89	36.71 ± 7.25	20.52 ± 1.21	0	0	0

Data are means ± Standard Deviation (SD) for six hamsters in each group. The formula V 4/3 (D1/2), (D2/2), and (D3/2), where D1, D2, and D3 are the three diameters (mm^3) of the tumour, was used to calculate the volume of the tumour. Values are presented as the mean SD for each set of 6 hamsters.

3.3 Histology

The histological alterations seen in experimental groups are shown in Table 2 and 3. Hyperkeratosis, epithelial hyperplasia, severe epithelial dysplasia, and well-differentiated OSCC were present in group 1. In group 2, hamsters showed moderate keratosis, hyperplasia mild dysplasia, and group 3 hamsters showed moderate keratosis, hyperplasia and mild dysplasia. In group 4, hamsters showed moderate hyperkeratosis, mild hyperplasia and in group 5, hamsters showed mild keratosis and hyperplasia. In group 6, hamsters showed standard cellular architecture without any indication of growth (Figure 2).

3.4 LPO By-Products

The levels of TBARS, CD, LOOH major LPO by-products in the buccal mucosa and plasma of each hamster group are shown in Figure 3. The tumour-bearing group 1 hamsters had significantly reduced levels of buccal mucosa LPO by-products, whereas group 1 hamsters had significantly greater levels in plasma ($p<0.05$). The DMBA-treated hamsters in groups 2 (AMS 100 mg/kg b.w.), 4 (AMS-NS 50 mg/kg b.w.), and 5 (AMS-NS 100 mg/kg b.w.) showed LPO by-product levels that were nearly normal, compared to the control.

3.5 Anti-Oxidant Status

Figure 4 shows the levels of enzymatic antioxidants, SOD, CAT, and GPx, nonenzymatic antioxidants, vitamin E, and GSH in the buccal mucosa and plasma of each group of hamsters. Antioxidant levels in the plasma of the tumour-bearing group 1 hamsters were dramatically reduced ($p<0.05$). In contrast, there was a major decrease

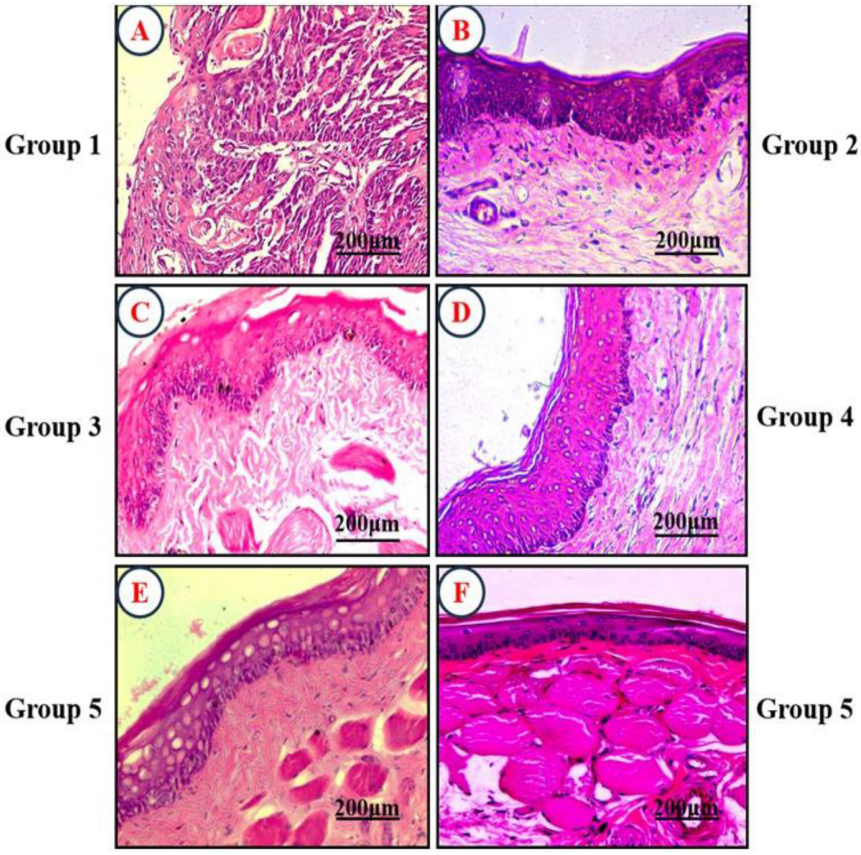


Figure 2. Histomorphology of hamsters in each category. **A)** Well-differentiated squamous cell carcinoma in group 1 hamsters that has epithelial and keratin pearls. **B)** Group 2 hamsters buccal pouch epithelium exhibited moderate keratosis, hyperplasia mild dysplasia. **C)** Group 3 hamsters buccal pouch epithelium exhibited moderate keratosis, hyperplasia and mild dysplasia. **D)** Hamster buccal pouch epithelium from group 4 showing moderate hyperkeratosis and mild hyperplasia. **E)** Hamster buccal pouch epithelium from group 5 showing mild keratosis and hyperplasia. In group 6 (Figure F), hamsters, standard cellular architecture without any indication of growth at 40x.

Table 3. Histopathological changes in the buccal mucosa of control and experimental hamsters in each group

Treatment	Keratosis	Hyperplasia	Dysplasia	Squamous Cell Carcinoma
DMBA	+++	+++	+++	+++
DMBA + AMS (100 mg/kg b.w.)	++	++	+	-
DMBA + AMS-NS (25 mg/kg b.w.)	++	++	+	-
DMBA + AMS-NS (50 mg/kg b.w.)	++	+	-	-
DMBA + AMS-NS (100 mg/kg b.w.)	+	+	-	-
Control	-	-	-	-

- = No change; + = mild; ++ = moderate; +++ = severe

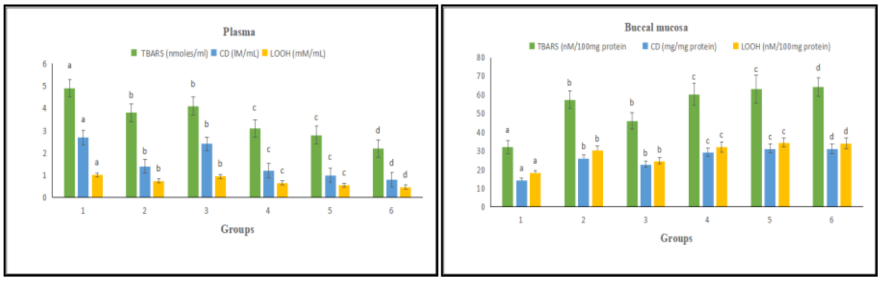


Figure 3. LPO byproduct levels (TBARS, CD, and LOOH) were evaluated in the plasma and buccal mucosa of hamsters from each group. The values are mean ± SD. Values without a specific superscript differ significantly ($p<0.05$).

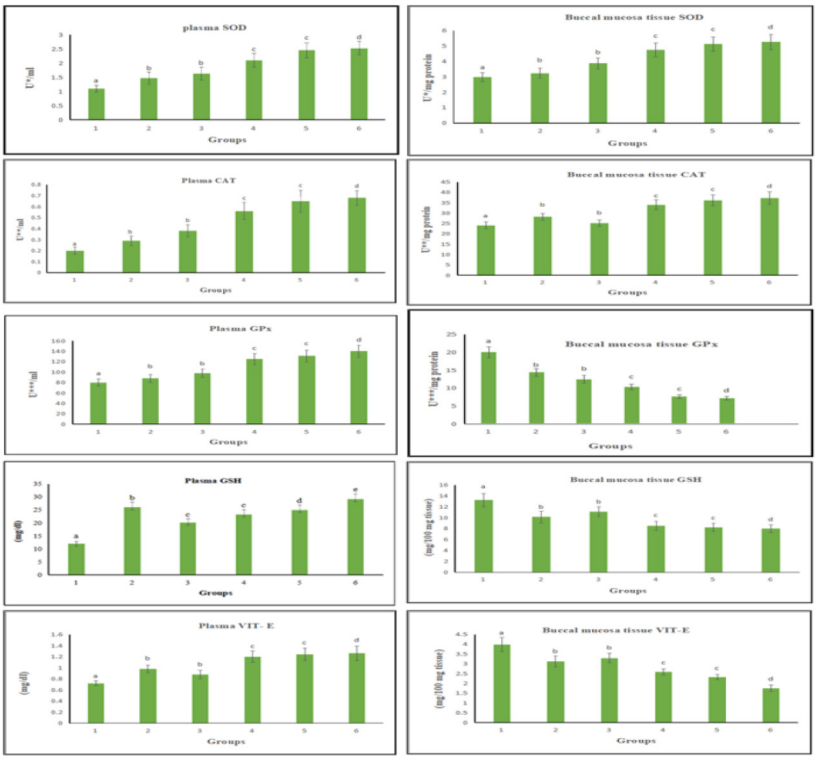


Figure 4. The value of enzymatic and non-enzymatic antioxidants in the plasma and buccal mucosa of hamsters from each group. U*: amount of enzyme to inhibit 50% NBT reduction/min. U**: µmole of H_2O_2 consumed/second. U#: µm of GSH consumed/min. The values are mean ± SD. Values without a specific superscript differ significantly ($p<0.05$).

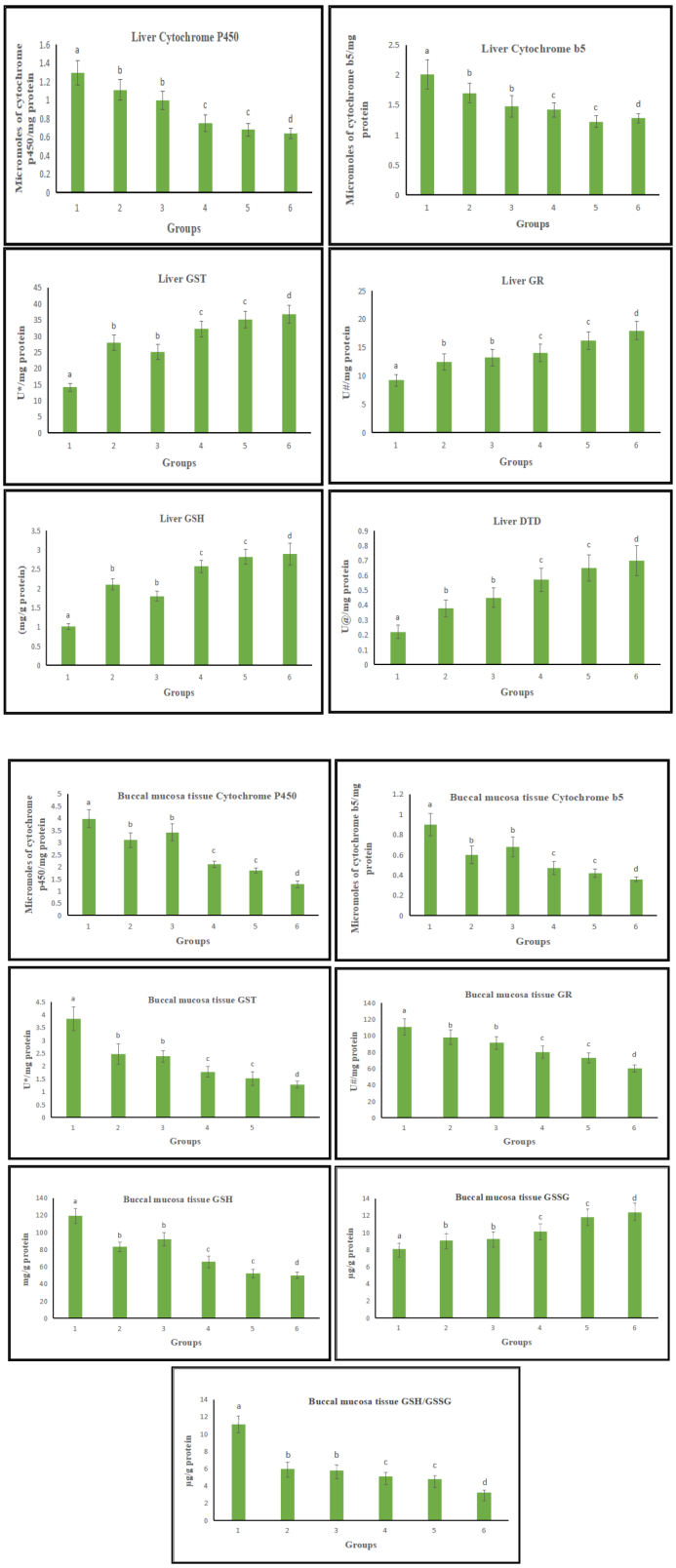


Figure 5. In each group, phase I and II detoxification enzyme activity in the hamster liver and buccal mucosa was determined. U*: micromoles of cytochrome p450; U#: l mole of H_2O_2 consumed/second. U@: l mole of glutathione peroxidase consumed/min. The values are mean ± SD. Values without a specific superscript differ significantly ($p<0.05$).

($p<0.05$) in SOD and CAT and an increase ($p<0.05$) in GPx, GSH, and vitamin E in the buccal mucosa of group 1 as compared to group 6. Antioxidants had returned to almost normal levels in DMBA and drug-treated group 2 (AMS 100 mg/kg b.w.), group 4 (AMS-NS 50 mg/kg b.w.), and group 5 (AMS-NS 100 mg/kg b.w.) hamsters.

3.6 Detoxification

The stage of phase I and phase II detoxification agents in the buccal mucosa and liver of each hamster group is shown in Figure 5. When tumour-bearing group 1 hamsters were the level of phase I (Cyt P450 and Cyt b5) detoxifying agents in the liver and buccal mucosa increased significantly ($p<0.05$) when compared with group 6. In liver levels of phase II detoxifying agents (GST, GR, DTD, and GSH) were dramatically reduced ($p<0.05$), and buccal mucosa levels of phase II detoxifying agents were altered (GST, GR, GSH, and GSH/GSSG increased, and GSSG decreased) in DMBA-treated hamsters, compared to group 6 hamsters. Oral administration of AMS and AMS-NS to DMBA-treated group 2 (AMS 100mg/kg b.w.), group 4 (AMS-NS 50mg/kg b.w.) and group 5 (AMS-NS 100mg/kg b.w.) hamsters returned the levels of phase I and II detoxification agents to near normal.

4. Discussion

DMBA, a polycyclic aromatic hydrocarbon, is frequently employed in experimental carcinogenesis and mutagenesis[31]. It is a well-known chemical carcinogen that causes persistent inflammation, DNA adduct formation, and ROS-mediated oxidative DNA damage in the buccal pouch, which encourages the development of cancer[32]. Researchers have often used DMBA-induced HBC models to evaluate the anticancer potential of both natural and synthetic compounds. Studies exploring the effectiveness of plant-derived chemicals as anticancer agents have revealed act on molecular targets associated with cancer progression. OSCs, which are sulfur-containing natural substances, play a key role in the prevention and treatment of various fatal illnesses, including cancer, diabetes, cardiovascular disease, neurological disorders, and infections[33]. Garlic contains several organosulfur compounds with health-promoting properties that exhibit antioxidant, anti-inflammatory, antibacterial, anti-hypertensive, anti-hyperlipidemic, and anti-diabetic properties[33]. Studies have demonstrated that the anti-cancer properties of OSCs are attributed to their ability to slow down the cell cycle, induce apoptosis, and regulate cell differentiation[34-37]. OSCs can effectively neutralise carcinogens and their metabolites, with a strong correlation observed between the length of the sulfur chain and their ability to suppress cancer cell growth[37]. Recent studies have shown that OSCs inhibit various types of cancers, including those of the prostate (Chandra-kuntal and Singh *et al*., 2010), pancreas (Ma *et al*., 2014), skin (Wang *et al*., 2012), colon (Lai *et al*., 2004), small intestine, stomach (Azarenko *et al*., 2014), and bladder (Munday *et al*., 2008)[38-43]. Cyclodextrin-diphenyl carbonate nanosponges represent a promising advancement in targeted cancer drug delivery, exploiting nanotechnology to address the limitations of conventional therapies. With a high surface area-to-volume ratio, they enhance drug selectivity for cancer cells, minimise toxicity to healthy tissues and overcome resistance mechanisms, ensuring safer and more effective treatments. Additionally, nanosponges, as versatile and biocompatible polymers, efficiently encapsulate a wide range of molecules while offering superior stability and nontoxicity.

AMS demonstrated significant chemopreventive potential in various cancers, although its specific effects on DMBA-induced oral carcinogenesis using nanosponges had not been previously investigated. In our study, hamsters treated with DMBA alone (Group 1) exhibited a 100% tumour incidence, developing well-differentiated Squamous Cell Carcinoma (SCC) within 14 weeks. This was accompanied by increased tumour volume and burden, significant weight loss, and reduced food intake. In contrast, treatment with AMS-NS significantly improved body weight in hamsters from groups 3 to 5, highlighting its chemopreventive efficacy. Consistent with prior studies on DMBA-induced oral carcinogenesis (Deng *et al*., 2021), histopathological analysis of group 1 revealed severe hyperkeratosis, hyperplasia, dysplasia and SCC development[44]. Notably, oral administration of AMS-NS suppressed the progression of keratosis, hyperplasia and dysplasia, effectively mitigating DMBA-induced buccal carcinogenesis and improving clinical outcomes. Due to the poor aqueous solubility of β-cyclodextrin-based AMS, to improve bioavailability and facilitate targeted delivery to cancer cells, leading to a suppression in histopathological features compared to the AMS-treated group 2 hamsters.

Cancer cells exhibited biological metabolites that differed significantly from those of normal cells. DMBA induced oral carcinogenesis in the buccal mucosa of hamsters through DNA adduct formation, chronic inflammation, and excessive production of reactive oxygen species[32]. This elevated ROS production led to oxidative modifications of nucleic acids, transforming normal cells into cancerous ones[45]. In our study, we observed decreased levels of LPO by-products in the tumour tissue of Group 1 hamsters, which were accompanied by chain oxidation of Polyunsaturated Fatty Acid (PUFAs), impairing membrane function and causing membrane oxidation. ROS further contributed to the oxidative modification of membrane lipids, generating their by-products such as Hydroperoxyl (HO^{2-}), Hydroxyl (HO^{-}), and Superoxide (O^{-}) radicals, among others[46]. The higher plasma LPO byproduct levels in DMBA-treated Group 1 hamsters likely resulted from damaged cancer tissues producing and releasing excess LPO byproducts, which subsequently leaked into the plasma[47]. This increase in LPO was linked to the overproduction of ROS and oxidative stress caused by DMBA, which was not observed in the normal groups. Our findings were consistent with earlier reports, as we observed higher levels of LPO byproducts in the plasma of DMBA-treated hamsters, while lower levels were found in the buccal mucosa. Administration of AMS-NS to groups 3-5 hamsters effectively restored the status of LPO byproduct levels in DMBA-induced hamster buccal pouch tumours. The protective outer coating of AMS-NS, composed of a glycopyranose-based sponge-like cavity structure, interacts easily with cancer cells via glucose-based receptor, when compared to the AMS-treated Group 2 hamsters, highlighting the therapeutic potential of AMS-NS in mitigating the oxidative damage caused by DMBA.

The carcinogen DMBA and its metabolites were shown to induce excessive production of ROS, leading to cellular membrane dysfunction, organelle damage, and nucleic acid base modifications, which contributed to mutagenesis and carcinogenesis compared to normal cells[48]. Elevated levels of free radicals, accompanied by reduced activity of key antioxidant enzymes such as SOD and CAT, were prominently observed in buccal mucosa tumour tissues due to oxidative stress. Our findings aligned with previous studies. Highlighting the excessive generation of ROS by NADPH oxidase, which triggers lipid peroxidation in response to cellular stress. This persistent imbalance favours disrupted normal antioxidant defence mechanisms, including enzymatic antioxidants (SOD, CAT, GPx) and non-enzymatic antioxidants (GSH, Vitamin E)[49]. Elevated levels of GSH, GPx, and Vitamin E were observed in DMBA-induced hamster buccal tumour tissues, playing a crucial regulatory role in cell survival, cellular proliferation and metastasis. Tumour cells sequestered these antioxidants to meet their biological and nutritional demands, supporting their rapid growth[50]. Treatment with AMS-NS in Groups 3–5 of DMBA-induced hamsters effectively restored antioxidant levels in buccal pouch tumours. AMS-NS, with its protective outer coating, sustained release, and excellent blood compatibility properties, demonstrated superior therapeutic potential compared to AMS alone (Group 2). These findings highlighted AMS-NS as a promising chemopreventive agent for managing oxidative stress and restoring redox homeostasis in DMBA-induced oral cancer.

Xenobiotics are detoxified by either eliminating reactive centres or conjugating them with an endogenous ligand to facilitate excretion[51]. The capacity to produce phase II agents without stimulating phase I enzymes appears to be a desirable property of a chemopreventive drug[52]. Chemopreventive drugs protect cells against carcinogenic intermediates by increasing phase II detoxification agents, specifically GST, GR, and GSH/GSSG, while suppressing phase I agents[53,54]. Organosulfur compounds can affect xenobiotic metabolism by inhibiting or inducing detoxification mechanisms[55]. In DMBA-treated hamsters, phase II detoxifying agents (GST, GR, GSH, and GSH/GSSG were increased and GSSG was decreased) active in the buccal mucosa, while decreasing in the liver when compared to control hamsters[56]. Our findings support the prior report. AMS-NS (Group 3-5) supplementation reduced metabolic activation of the carcinogen during DMBA-induced hamster buccal pouch carcinogenesis by restoring detoxifying agents (phase I and II enzymes) in both liver and buccal mucosal tissues.

This study suggests that the chemopreventive and dose-dependent properties of AMS-NS effectively suppressed tumour growth in the buccal mucosa by significantly reducing abnormal cell proliferation and histopathological modifications. This may be achieved through its antioxidant activity, suppression of LPO, and modulation of detoxification agents in DMBA-induced oral carcinogenesis. However, further research is needed to fully elucidate the molecular mechanisms underlying the chemopreventive efficacy of AMS-NS (50 mg/kg

body weight) in DMBA-induced hamster buccal pouch carcinogenesis.

5. Conclusion

This study developed an AMS-NS system that enhanced the pharmacological properties of AMS. These findings revealed that the chemopreventive capacity of AMS-NS effectively inhibited tumour growth in the buccal mucosa by significantly reducing abnormal cell proliferation in DMBA-induced oral squamous cell carcinomas. The protective effects of AMS-NS were attributed to its ability to enhance antioxidant enzyme activity, modulate detoxifying agents and markedly reverse lipid peroxidation levels. These results suggest that oral administration of AMS-NS at doses of 50 and 100 mg/kg body weight was a potentially chemopreventive approach. Consequently, the AMS-NS formulation based on beta-cyclodextrin and nanosponge technology was proposed as a promising carrier for the effective treatment and prevention of oral cancer. Further research was warranted to explore its therapeutic applications and mechanisms in greater detail.

6. Author Contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Saranya Jawaharlal, Veerasamy Vinothkumar, Duraisamy Ramachandhiran, Sankaranarayanan Chandrasekaran, Srinivasan Subramani, and Geetha Devarajan. The first draft of the manuscript was written by Saranya Jawaharlal, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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8. Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

9. Acknowledgement

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10. References

1. Nagai H, Kim YH. Cancer prevention from the perspective of global cancer burden patterns. J Thorac Dis. 2017; 9(3):448-451. https://doi.org/10.21037/jtd.2017.02.75 PMid:28449441 PMCid:PMC5394024
2. Al-Afifi NA, Alabsi AM, Shaghayegh G, Ramanathan A, Ali R, Alkoshab M, Bakri MM. The *in vitro* and *in vivo* antitumor effects of *Dracaena cinnabari* resin extract on oral cancer. Arch Oral Biol. 2019; 104:77-89. https://doi.org/10.1016/j.archoralbio.2019.05.030 PMid:31176147
3. Krishna Rao SV, Mejia G, Roberts-Thomson K, Logan R. Epidemiology of oral cancer in Asia in the past decade-an update (2000-2012). Asian Pac J Cancer Prev. 2013; 14(10):5567-5577. https://doi.org/10.7314/APJCP.2013.14.10.5567 PMid:24289546.
4. Sathishkumar K, Chaturvedi M, Das P, Stephen S, Mathur P. Cancer incidence estimates for 2022 and projection for 2025: Result from National Cancer Registry Programme, India. Indian J Med Res. 2022; 156(4 and 5):598-607. https://doi.org/10.4103/ijmr.ijmr_1821_22 PMid:36510887 PMCid:PMC10231735.
5. Silvan S, Manoharan S. Apigenin prevents deregulation in the expression pattern of cell-proliferative, apoptotic, inflammatory and angiogenic markers during 7, 12-dimethylbenz [a] anthracene-induced hamster buccal pouch carcinogenesis. Arch Oral Biol. 2013; 58(1):94-101. https://doi.org/10.1016/j.archoralbio.2012.06.005 PMid:22776109.
6. Abel EL, Angel JM, Kiguchi K, DiGiovanni J. Multi-stage chemical carcinogenesis in mouse skin: Fundamentals and applications. Nat Protoc. 2009; 4(9):1350-1362. https://doi.org/10.1038/nprot.2009.120 PMid:19713956 PMCid:PMC3213400.
7. Mohammed AI, Sangha S, Nguyen H, Shin DH, Pan M, Park H, McCullough MJ, Celentano A, Cirillo N. Assessment of oxidative stress-induced oral epithelial toxicity. Biomolecules. 2023; 13(8):1239. https://doi.org/10.3390/biom13081239 PMid:37627304 PMCid:PMC10452318.
8. Ramshankar V, Krishnamurthy A. Chemoprevention of oral cancer: Green tea experience. J Nat Sci Biol Med. 2014; 5(1):3-7. https://doi.org/10.4103/0976-9668.127272 PMid:24678188 PMCid:PMC3961947.
9. Khazir J, Mir BA, Pilcher L, Riley DL. Role of plants in anticancer drug discovery. Phytochem Lett 7. 2014; 173-181. https://doi.org/10.1016/j.phytol.2013.11.010

10. Steward WP, Brown K. Cancer chemoprevention: A rapidly evolving field. Br J Cancer. 2013; 109(1):1-7. https://doi.org/10.1038/bjc.2013.280 PMid:23736035 PMCid:PMC3708589.
11. Rose P, Whiteman M, Moore PK, Zhu YZ. Bioactive S-alk(en)yl cysteine sulfoxide metabolites in the genus Allium: The chemistry of potential therapeutic agents. Nat Prod Rep. 2005; 22(3):351-368. https://doi.org/10.1039/b417639c PMid:16010345.
12. Amagase H, Petesch BL, Matsuura H, Kasuga S, Itakura Y. Intake of garlic and its bioactive components. J Nutr. 2001; 131(3s):955S-962S. https://doi.org/10.1093/jn/131.3.955S PMid:11238796.
13. Becker PM, van Wikselaar PG, Mul MF, Pol A, Engel B, Wijdenes JW, van der Peet-Schwering CM, Wisselink HJ, Stockhofe-Zurwieden N. *Actinobacillus pleuropneumoniae* is impaired by the garlic volatile Allyl Methyl Sulfide (AMS) *in vitro* and in-feed garlic alleviates pleuropneumonia in a pig model. Vet Microbiol. 2012; 154(3-4):316-324. https://doi.org/10.1016/j.vetmic.2011.07.011 PMid:21824734.
14. Wargovich MJ. Diallylsulfide and allylmethylsulfide are uniquely effective among organosulfur compounds in inhibiting CYP2E1 protein in animal models. J Nutr. 2006; 136(3 Suppl):832S-834S. https://doi.org/10.1093/jn/136.3.832S PMid:16484575.
15. Torne S, Darandale S, Vavia P, Trotta F, Cavalli R. Cyclodextrin-based nanosponges: Effective nanocarrier for tamoxifen delivery. Pharm Dev Technol. 2013; 18(3):619-625. https://doi.org/10.3109/10837450.2011.649855 PMid:22235935.
16. Jawaharlal S, Subramanian S, Palanivel V, Devarajan G, Veerasamy V. Cyclodextrin-based nanosponges as promising carriers for active pharmaceutical ingredient. J Biochem Mol Toxicol. 2024; 38(1):e23597. https://doi.org/10.1002/jbt.23597 PMid:38037252.
17. Swaminathan S, Cavalli R, Trotta F, Ferruti P, Ranucci E, Gerges I, Manfredi A, Marinotto D, Vavia PR. *In vitro* release modulation and conformational stabilization of a model protein using swellable polyamidoamine nanosponges of β-cyclodextrin. Eur J Pharm Biopharm. 2010; 74(2):193-201. https://doi.org/10.1016/j.ejpb.2009.11.003 PMid:19900544.
18. Swaminathan S, Cavalli R, Trotta F. Cyclodextrin-based nanosponges: A versatile platform for cancer nanotherapeutics development. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2016; 8(4):579-601. https://doi.org/10.1002/wnan.1384 PMid:26800431.
19. Cui F, Shi CL, Zhou XJ, Wen W, Gao XP, Wang LY, He B, Yin M, Zhao JQ. *Lycium barbarum* Polysaccharide extracted from *Lycium barbarum* leaves ameliorates asthma in mice by reducing inflammation and modulating gut microbiota. J Med Food. 2020; 23(7):699-710. https://doi.org/10.1089/jmf.2019.4544 PMid:32392444.
20. Pandey P, Khan F, Alshammari N, Saeed A, Aqil F, Saeed M. Updates on the anticancer potential of garlic organosulfur compounds and their nanoformulations: Plant therapeutics in cancer management. Front Pharmacol. 2023; 14:1154034. https://doi.org/10.3389/fphar.2023.1154034 PMid:37021043 PMCid:PMC10067574.
21. Shklar G, Schwartz JL. Vitamin E inhibits experimental carcinogenesis and tumour angiogenesis. Eur J Cancer B Oral Oncol. 1996; 32B(2):114-119. https://doi.org/10.1016/0964-1955(95)00077-1 PMid:8736173.
22. Ribeiro FC, Silva JC, Santos JL, Pontes KC. Diagnóstico da pneumonia enzoótica suína pela técnica da imunoperoxidase. Arq Bras Vet Zootec. 2004; 709-714. https://doi.org/10.1590/S0102-09352004000600003
23. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys. 1984; 21(2):130-132.
24. Sinha AK. Colorimetric assay of catalase. Anal Biochem. 1972; 47(2):389-394. https://doi.org/10.1016/0003-2697(72)90132-7 PMid:4556490.
25. Beutler E, Kelly BM. The effect of sodium nitrite on red cell GSH. Experientia. 1963; 19:96-97. https://doi.org/10.1007/BF02148042 PMid:13967892.
26. Jiang ZY, Hunt JV, Wolff SP. Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low-density lipoprotein. Anal Biochem. 1992; 202(2):384-389. https://doi.org/10.1016/0003-2697(92)90122-N PMid:1519766.
27. Rao KS, Recknagel RO. Early onset of lipoperoxidation in rat liver after carbon tetrachloride administration. Exp Mol Pathol. 1968; 9(2):271-278. https://doi.org/10.1016/0014-4800(68)90041-5 PMid:4952076.
28. Omura T, Sato T. The carbon monoxide-binding pigment of liver microsomes. i. Evidence for its hemoprotein nature. J Biol Chem. 1964;239:2370-2378. https://doi.org/10.1016/S0021-9258(20)82244-3 PMid:14209971.
29. Ernster L. DT diaphorase. Meth Enzymol. 1967; 10:309-317. https://doi.org/10.1016/0076-6879(67)10059-1
30. Lowry OH, Rosebrough NJ, Farr Al, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951; 193(1):265-275. https://doi.org/10.1016/S0021-9258(19)52451-6 PMid:14907713.
31. Zhang L, Sun D, Bao Y, Shi Y, Cui Y, Guo M. Nerolidol protects against LPS-induced acute kidney injury via inhibiting TLR4/NF-κB signalling. Phytother Res. 2017; 31(3):459-465. https://doi.org/10.1002/ptr.5770 PMid:28093813.
32. Wani MK, Yarber RH, Ahmed A, Hengesteg A, Robbins KT. Cancer induction in the DMBA hamster cheek pouch: A modified technique using a promoter. Laryngoscope. 2001; 111(2):204-206. https://doi.org/10.1097/00005537-200102000-00003 PMid:11210861.

33. Walag AM, Ahmed O, Jeevanandam J, Akram M, Ephraim-Emmanuel BC, Egbuna C, Semwal P, Iqbal M, Hassan S, Uba JO. Health benefits of organosulfur compounds. In Functional foods and nutraceuticals: Bioactive components, formulations and innovations. Cham: Springer International Publishing. 2020. pp. 445-472. https://doi.org/10.1007/978-3-030-42319-3_21
34. Xiao D, Choi S, Johnson DE, Vogel VG, Johnson CS, Trump DL, Lee YJ, Singh SV. Diallyl trisulfide-induced apoptosis in human prostate cancer cells involves c-Jun N-terminal kinase and extracellular-signal-regulated kinase-mediated phosphorylation of Bcl-2. Oncogene. 2004; 23(33):5594-5606. https://doi.org/10.1038/sj.onc.1207747 PMid:15184882.
35. Xiao D, Herman-Antosiewicz A, Antosiewicz J, Xiao H, Brisson M, Lazo JS, Singh SV. Diallyl trisulfide-induced G(2)-M phase cell cycle arrest in human prostate cancer cells is caused by reactive oxygen species-dependent destruction and hyperphosphorylation of Cdc 25 C. Oncogene. 2005; 24(41):6256-6268. https://doi.org/10.1038/sj.onc.1208759 PMid:15940258.
36. Herman-Antosiewicz A, Singh SV. Checkpoint kinase 1 regulates diallyl trisulfide-induced mitotic arrest in human prostate cancer cells. J Biol Chem. 2005; 280(31):28519-28528. https://doi.org/10.1074/jbc.M501443200 PMid:15961392.
37. Lanzotti V, Scala F, Bonanomi G. Compounds from Allium species with cytotoxic and antimicrobial activity. Phytochem Rev. 2014; 769-791. https://doi.org/10.1007/s11101-014-9366-0
38. Cerella C, Dicato M, Jacob C, Diederich M. Chemical properties and mechanisms determining the anti-cancer action of garlic-derived organic sulfur compounds. Anticancer Agents Med Chem. 2011; 11(3):267-271. https://doi.org/10.2174/187152011795347522 PMid:21269260.
39. Chandra-Kuntal K, Singh SV. Diallyl trisulfide inhibits activation of signal transducer and activator of transcription 3 in prostate cancer cells in culture and in vivo. Cancer Prev Res (Phila). 2010; 3(11):1473-1483. https://doi.org/10.1158/1940-6207.CAPR-10-0123 PMid:20959517 PMCid:PMC2988081.
40. Ma HB, Huang S, Yin XR, Zhang Y, Di ZL. Apoptotic pathway induced by diallyl trisulfide in pancreatic cancer cells. World J Gastroenterol. 2014; 20(1):193-203. https://doi.org/10.3748/wjg.v20.i1.193 PMid:24415872 PMCid:PMC3886008.
41. Wang HC, Pao J, Lin SY, Sheen LY. Molecular mechanisms of garlic-derived allyl sulfides in the inhibition of skin cancer progression. Ann N Y Acad Sci. 2012; 1271(1):44-52. https://doi.org/10.1111/j.1749-6632.2012.06743.x PMid:23050963 PMCid:PMC3499657.
42. Lai PK, Roy J. Antimicrobial and chemopreventive properties of herbs and spices. Curr Med Chem. 2004; 11(11):1451-1460. https://doi.org/10.2174/0929867043365107 PMid:15180577.
43. Azarenko O, Jordan MA, Wilson L. Erucin, the major isothiocyanate in arugula (*Eruca sativa)*, inhibits proliferation of MCF7 tumour cells by suppressing microtubule dynamics. PLoS One. 2014; 9(6):e100599. https://doi.org/10.1371/journal.pone.0100599 PMid:24950293 PMCid:PMC4065051.
44. Munday R, Mhawech-Fauceglia P, Munday CM, Paonessa JD, Tang L, Munday JS, Lister C, Wilson P, Fahey JW, Davis W, Zhang Y. Inhibition of urinary bladder carcinogenesis by broccoli sprouts. Cancer Res. 2008; 68(5):1593-1600. https://doi.org/10.1158/0008-5472.CAN-07-5009 PMid:18310317.
45. Deng Y, Ma M, Guo G, Tang Z. Kirenol regulates the cell proliferative and inflammatory markers in DMBA-induced oral squamous cell carcinogenesis in hamster. Environ Toxicol. 2021; 36(3):328-338. https://doi.org/10.1002/tox.23039 PMid:33044773.
46. Abiaka C, Al-Awadi F, Al-Sayer H, Gulshan S, Behbehani A, Farghally M, Simbeye A. Serum antioxidant and cholesterol levels in patients with different types of cancer. J Clin Lab Anal. 2001; 15(6):324-330. https://doi.org/10.1002/jcla.1045 PMid:11793433 PMCid:PMC6807942.
47. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signalling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev. 2014; 2014:360438. https://doi.org/10.1155/2014/360438 PMid:24999379 PMCid:PMC4066722.
48. Yang K, Zhang G, Mei J, Chen D, Wu M. Screening and analysis of pathogenic genes during DMBA-induced buccal mucosa carcinogenesis in golden hamsters. Oncol Rep. 2010; 23(6):1619-1624. https://doi.org/10.3892/or_00000803
49. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J. 2012; 5(1):9-19. https://doi.org/10.1097/WOX.0b013e3182439613 PMid:23268465 PMCid:PMC3488923.
50. Gach K, Długosz A, Janecka A. The role of oxidative stress in anticancer activity of sesquiterpene lactones. Naunyn Schmiedebergs Arch Pharmacol. 2015; 388(5):477-486. https://doi.org/10.1007/s00210-015-1096-3 PMid:25656627.
51. George S, Abrahamse H. Redox potential of antioxidants in cancer progression and prevention. Antioxidants (Basel). 2020; 9(11):1156. https://doi.org/10.3390/antiox9111156 PMid:33233630 PMCid:PMC7699713.

52. Hanlon N, Okpara A, Coldham N, Sauer MJ, Ioannides C. Modulation of rat hepatic and pulmonary cytochromes P450 and phase II enzyme systems by erucin, an isothiocyanate structurally related to sulforaphane. J Agric Food Chem. 2008; 56(17):7866-7871. https://doi.org/10.1021/jf801456h PMid:18680373.
53. Vahora H, Khan MA, Alalami U, Hussain A. The potential role of nitric oxide in halting cancer progression through chemoprevention. J Cancer Prev. 2016; 21(1):1-12. https://doi.org/10.15430/JCP.2016.21.1.1 PMid:27051643 PMCid:PMC4819660.
54. Cheung KL, Kong AN. Molecular targets of dietary phenethyl isothiocyanate and sulforaphane for cancer chemoprevention. AAPS J. 2010; 12(1):87-97. https://doi.org/10.1208/s12248-009-9162-8 PMid:20013083 PMCid:PMC2811646.
55. Swanson HI, Njar VC, Yu Z, Castro DJ, Gonzalez FJ, Williams DE, Huang Y, Kong AN, Doloff JC, Ma J, Waxman DJ, Scott EE. Targeting drug-metabolising enzymes for effective chemoprevention and chemotherapy. Drug Metab Dispos. 2010; 38(4):539-544. https://doi.org/10.1124/dmd.109.031351 PMid:20233842 PMCid:PMC2845935.
56. Melino S, Sabelli R, Paci M. Allyl sulfur compounds and cellular detoxification system: effects and perspectives in cancer therapy. Amino Acids. 2011; 41(1):103-112. https://doi.org/10.1007/s00726-010-0522-6 PMid:20213447.
57. Ramachandhiran D, Vinothkumar V, Babukumar S. Paeonol exhibits anti-tumour effects by apoptotic and anti-inflammatory activities in 7,12-dimethylbenz(a) anthracene-induced oral carcinogenesis. Biotech Histochem. 2019; 94(1):10-25. https://doi.org/10.1080/10520295.2018.1493221 PMid:30101628.