Research Article

Yi Gan, Ai'e He, Lilei Zhu, Yao Yao and Chunhua Lv*

Lycorine impedes 7,12-dimethylbenz(a) anthracene exposed hamster oral carcinogenesis through P13K/Akt and NF-κB inhibition

Abstract

Objectives: Oncogenic signaling pathways that are activated abnormally play a key activity in tumor initiation and development. This research aimed to examine the preventive efficiency of lycorine in the buccal pouch hamster tumor model based on its capacity to target phosphoinositide 3-kinase (PI3K)/Akt and nuclear factor-kappa B (NF-κB) signaling cascades.

Methods: The induction of oral tumor in male golden Syrian hamsters was done by 7,12-dimethylbenz [a] anthracene (DMBA) painting on the left buccal pouch thrice a week for 10 weeks. The chemopreventive effect of lycorine (20 mg/kg b.w.) was assessed by treating orally for 14 weeks of the experimental period. The biochemical endpoints such as lipid peroxidation (LPO), antioxidants, and phase I and II detoxification agents were analyzed.

Results: The treatment of lycorine to DMBA-induced hamsters drastically suppressed tumor incidence and tumor size and reverted the levels of the biochemical indicator. Moreover, lycorine significantly downregulated the p53, Cyclooxygenase 2 (cox-2), and P13K/Akt signaling and inhibited the phosphorylation of NF-κB and nuclear factor-kappa-B-inhibitor alpha (Iκ-Bα) in DMBA-induced hamsters.

Conclusions: The oral administration of lycorine effectively inhibited tumor cell proliferation, restored the antioxidant, LPO, and detoxification enzymes, and inhibited NF-κB signaling in oral tumorigenesis. Thus, the use of lycorine after a proper clinical trial could be effective for oral tumorigenesis treatment.

Keywords: buccal pouch carcinogenesis; detoxification agents; lycorine; NF-κB signaling; P13K/Akt signaling.

Introduction

Oral tumor is the most prevalent kind of cancer with a deprived prognosis, affecting over 0.5 million new cases every year worldwide [1]. Commonly, 90% of oral tumors develop in the oral squamous epithelium. Oral squamous cell cancer is the most predominant cancer with a high incidence rate, and it accounts for around a third of all malignancies in southeast regions of Asia [2]. Although modern chemotherapy methods can suppress tumor growth in several ways, they are usually associated with significant side effects. Even in modern medicine, the drugs against oral cancer are typically natural substances. The discovery of a drug with fewer side effects, a credible strategy for reducing oral cancer, could be helpful. The 7,12-dimethylbenz [a] anthracene (DMBA) is a carcinogen that creates deoxyribonucleic acid, which damages nuclear material and eventually causes mutations. Human malignant tumors of the DMBA-induced oral mucosal cancer type are the most acceptable and often utilized models [3].

Yi Gan and Ai'e He contributed equally to this work.

*Corresponding author: Chunhua Lv, Department of Stomatology, Taizhou People's Hospital, Taizhou, Jiangsu, 225300, P.R. China, E-mail: lvchunhua095@sina.com. https://orcid.org/0000-0001-7920-509X
Yi Gan, Department of Dental, Sixth People's Hospital of Chengdu, Sichuan, Chengdu, P.R. China. https://orcid.org/0000-0003-0644-3921
Ai'e He, Department of Stomotology, Wuhan No. 5 Hospital, Wuhan, Hubei, P.R. China. https://orcid.org/0000-0002-4146-9468
Lilei Zhu, Department of Periodontology and Oral Medicine, Changsha Stomatological Hospital, Changsha, Hunan, P.R. China. https://orcid.org/0000-0002-0745-2777
Yao Yao, Department of Orthodontics, Changsha Stomatological Hospital, Changsha, Hunan, P.R. China. https://orcid.org/0000-0002-9127-5856

In the metabolism of carcinogens, the liver plays a crucial role. Phase I detoxifying agents catalyze carcinogen metabolic stimulation, with the resulting cancer-causing metabolites released by phase II detoxifying markers glutathione S-transferase (GST) and glutathione reductase (GR) via conjugation of reduced glutathione glucose (GSH) acid. The enzymes Cytochromes P_{450} (cyt-P_{450}) and Cytochrome $b5$ (cyt-b_5) are engaged in the metabolic stimulation of DMBA. Mcfadyen et al. [4], reported that cyt-P450 enzymes transform lipophilic agents into polar metabolites, which are subsequently played on by phase II enzymatic agents like GST and GR, strengthening their divergence and facilitating their excretion. Microsomal cyt-b5 is a hemoprotein involved in metabolic activities, including fatty acid desaturation, the generation of steroid hormones, and the decrease of methemoglobin. GSTs are a family of enzymes that help remove reactive metabolites by accelerating the coupling of reactive oxygen species (ROS) to GSH [5]. GSH levels in cells are maintained by GR, which catalysis the reduced nicotinamide adenine dinucleotide phosphate (NADPH)-mediated decrease of glutathione disulfide to glutathione [6]. DT-diaphorase (DTD) is an electron transfer flavoprotein that uses quinine as a hydrogen acceptor to oxidize NADH or NADPH to NAD^+ or $NADP^+$. DTD plays an essential role in defending the cells from oxidative injury by averting the establishment of oxyradicals [7]. The condition of phase I and phase II detoxifying mediators in the liver can be used to determine a test compound's chemopreventive capability.

The phosphatidylinositol-3-kinase (PI3K)/Akt/pathway is the most commonly regulated molecular pathway, and it's often used as a cancer balancer and involved in divers of cellular physiological events [8]. Several studies have found that the PI3K/Akt pathway downregulation is linked to the activation and development of various malignancies, particularly oral cancer. In many cancers, aberrant stimulation of the PI3K/Akt pathway is involved in chemotherapy drug resistance [9]. Since abnormal activation of the NF-κB is involved in cellular processes, it's vital to comprehend the significance of NF-B proteins, especially during the advancement and spread of numerous malignancies, especially oral cancer. The NF-κB was preferentially activated in cancers such as oral, breast, pancreatic, and colon cancers [10]. The NF-κB signaling is primarily regulated by the interaction of NF-κB complexes with their inhibitors, IκB proteins. P13K/Akt signaling is well recognized for triggering NF-kB by phosphorylating and degrading the inhibitory component IκB [11].

Because of their non-toxicity and absence of side effects, natural substances represent a rich supply and potential of anti-carcinogenic drugs today. Due to apoptosis induced in cancer cells, a significant variety of medicinal plants has been examined in favor of the anti-carcinogenic effect. Lycorine is an alkaloid compound isolated from the genera Amaryllidaceae. The antiproliferative, anti-lipid peroxidant, anti-inflammatory, antirheumatic, neuroprotective [12], and immunomodulatory actions are pharmacological properties of lycorine. Moreover, lycorine suppressed the progression of leukemia and myeloma [13]. However, by analyzing its regulating influence on the modulation of molecular markers connected with inflammation, and death during cell carcinogenesis, the molecular processes underlying the anti-tumor potential of lycorine can be discovered. This research aimed to appraise the anti-tumor efficiency of lycorine on oral tumorigenesis in hamster buccal pouches. These studies may aid in the discovery of a new chemopreventive medication for oral tumors.

Materials and methods

Chemicals

Lycorine (purity ≥ 98%, CAS: 2188-68-3) and DMBA (purity ≥ 95%, CAS: 57-97-6) were acquired from Sigma-Aldrich. Biovision, CA, USA. All other analytical grade chemicals and solvents utilized in the experiments were procured Hi-Media Laboratories Pvt. Ltd. China.

Animals

Male golden Syrian (*Mesocricetus auratus*) hamsters weighing 80–120 g were bought and kept in a protected environment of 22–25 °C and 45–55% humidity. Hamsters were alienated in sanitized polypropylene cages with 12 h of light/dark cycles, water, and regular pellet food under normal conditions. They have enough room to divide their quarters in a cage. Their environmental enrichment will be enhanced by more space or two contiguous rooms. For a hamster, systems cages give a good incentive. All the experimental works were approved by Chengdu Sixth People's Hospital ethical committee (Approval Number: 202000197).

Experimental design

The schematic presentation of the design of the experiment was presented (Supplementary Figure 1). The hamsters were alienated into four groups of six hamsters. The group I animals were fed a pellet diet for 14 weeks and designated as the control group. For 10 weeks, Group II animals were given pellet and topical painting of DMBA (0.5%/kg b.w.) on the buccal pouch of the left thrice for a week. Group III animals were fed a pellet diet containing lycorine (20 mg/kg b.w.) through oral gavage three times a week on days other than DMBA induction, a week before DMBA application, and continued for 14 weeks. Group IV animals received a pellet diet with

lycorine (20 mg/kg b.w.) alone through oral gavage three times a week for 14 weeks. The experimentation was completed after the 14th week, and all the animals were killed by cervical displacement. The left buccal mucosa was inspected and photographed before the hamsters were killed and examined for tumor formation. Before weighing, the left cheek pouches were collected and promptly blotted dry.

Drug preparation and DMBA induction

Lycorine was prepared by dissolving in PBS with 5% DMSO and given orally to the relevant animals. The left buccal pouches of animals were applied three times a week with a 0.5% DMBA in liquid paraffin solution, applied with a number four brush.

Tumor analysis

The total tumors formed in the cheek pouch of hamsters were inspected, and the size of the tumor was calculated using a scale after the hamsters were euthanized. The tumor volume was calculated using formula V=4/3 (D1/2) (D2/2) (D3/2), where D1, D2, and D3 are the three diameters (mm^3) of the tumor, respectively, as reported by [14]. The tumor burden was determined by calculating the tumor volume with the tumors counts in each group.

Histological studies

The dissected cheek pouches were placed in 10% formalin, mounted in paraffin, and thin sections were made. The sections were inflated with hot water and loaded onto slides with care. Hematoxylin and eosin dye was also utilized to stain the sections. The sections were examined for histopathological changes after staining, and photomicrographs were obtained with a light microscope fitted with a digital camera at 40× magnification.

Preparation of plasma and tissue samples

Heparinized tubes were used to obtain the blood samples from the jugular veins of hamsters. By centrifuging the blood samples at 1000 × gravity for 15 min, the plasma was recovered. The liver; and buccal mucosa were collected and homogenized in a suitable buffer with a mortar and pestle and utilized for biochemical assays.

Measurement of detoxifying agents

The Cyt P450 and Cyt b5 detoxifying enzymes in the buccal and liver were examined by the method recommended by Omura and Sato [15]. The level of GST in the liver and buccal mucosa was determined by reacting the homogenate samples with 1-chloro-2,4-dinitrobenzene and recording Optical Density (OD) at 540 nm [16]. After reaction with the addition of GSH, the OD was recorded at 540 nm. The activity of GR was assessed by the method of Carlberg and Mannervik [17]. The levels of the liver- DTD were determined using Ernster's [18] method, which involved measuring at 550 nm with NADPH.

Measurement of LPO, enzymatic and non-enzymatic antioxidants

The concentrations of thiobarbituric acid reactive substances (TBARS) in plasma and buccal mucosa were measured using the Yagi [19] and Ohkawa et al. [20], methods, respectively. The levels of superoxide dismutase, catalase, and glutathione peroxidase were measured using the methods of Kakkar et al. [21], Sinha [22], and Rotruck et al. [23]. The method based on the classical Emmerie Engle reaction was used to quantify vitamin E in plasma [24]. The vitamin E concentration in the tissues was calculated by adopting the Desai et al. [25], technique.

Western blot analysis

Frozen hamster cheek pouches were homogenized using ice-cold lysis buffer (Wuhan Servicebio Technology Co., Ltd.). The BCA technique was used to determine protein concentrations, as indicated by the manufacturer (Wuhan Service bio Technology Co., Ltd.). The proteins were isolated by 15% SDS-PAGE (Beijing Liuyi Instrument Factory). Following this, the protein was transferred by wet transfer into nitrocellulose membranes (PVDF; Millipore, Billerica, MA, USA). Further, the membrane was probed at ambient temperature with 5% BSA for 2 h before reacting with the primary antibodies overnight at 4 °C. The antibodies p53 (#9282), Caspase 9 (#9508), Cox-2 (#4842), PI3K (#4257), Akt (#4691), p-Akt (#4060), NF-κB (#9936), p-NF-κB (#3033), IκB (#4814), p-IκB (2859), and GAPDH (#5174) purchased from Cell Signaling Technology, Denver, MA., USA were used at a dilution of 1:1000. Finally, the protein bands were exposed for 3–10 min to the chemiluminescent reagent (ECL). Fluorescence was taken on X-ray photographic film in a dark room to obtain the expressions of all the proteins. The band densities of the above proteins were quantitatively examined using Image J software (NIH, Bethesda, MD, United States).

Statistical analyses

The mean ± standard deviation (SD) of three replicates was used to display all of the results. The one-way ANOVA was followed by Tukey's post hoc test for multiple comparisons between groups using Prism version 5 (GraphPad Software, Inc.). If the p-value ≤ 0.05, the results were measured significant statistically.

Results

Effect of lycorine on tumor incidence, number, volume, burden

All the hamsters were checked for the total count of tumors, tumor incidence, number, volume, and burden. We noticed 100% tumor development in the DMBA painted carcinogenic group, with a mean tumor volume of 164.32 ± 9.76 mm^3 and a tumor burden of 1,167.65 ± 59.63 mm^3. In DMBA-painted hamsters, the treatment of lycorine (20 mg/kg b.w.) significantly reduced the development of tumors. Untreated control hamsters (group 1)

Table 1: Lycorine prevents DMB exposed tumor incidence, tumor volume, and tumor burden in a hamster model.

	Control	DMBA	DMBA + lycorine (20 mg/kg b.w.)	Lycorine alone (20 mg/kg b.w.)
Tumor incidence	0%	100%	0	0
Total number of tumor/hamsters models	0	9.54 ± 0.94	0	0
Total volume (mm^3)/hamster model	0	164.32 ± 9.76	0	0
Tumor burden (mm^3)/hamster model	0	1167.65 ± 59.63	0	0

Value of the data were articulated in mean ± standard deviation (n=6).

and hamsters given lycorine alone did not develop tumors (group 4) (Table 1).

Lycorine induced histopathological changes in buccal mucosa

All hamsters' buccal mucosa was investigated for abnormal changes. We noticed significant hyperplasia, dysplasia, and well-differentiated squamous cell carcinoma in DMBA-induced hamsters. Although, in the DMBA-painted and lycorine (20 mg/kg b.w.) treated rats, the severity of hyperplasia, dysplasia, and squamous cell carcinoma in the buccal mucosa was reduced. The epithelial layers of hamsters given lycorine (20 mg/kg b.w.) alone were well defined and unharmed, similar to control hamsters (Figure 1A).

Effects of lycorine on phase I and II detoxicates in liver

In tumor-bearing hamsters, phase I (cyt-P450 and cyt-*b5*) enzymes were markedly elevated, while the amounts of phase II liver function enzymes (GSH, GST, GR, and DTD) were dramatically reduced. In tumor-bearing hamsters (group III), oral lycorine treatment drastically reduced phase I agents while raising phase II agents to near-normal levels (Group III). When hamsters in groups IV and I were compared, there was no evident alteration in the levels of liver phase I and II functional enzymes (Figure 1B).

Effects of lycorine on phase I and II detoxicates in buccal mucosa

The phase I and II enzymes in buccal mucosa were notably increased in DMBA painted mucosal carcinogenesis group II hamsters than in control hamsters. The lycorine oral treatment to DMBA-induced hamsters effectively reduced the phase I and II agents to levels close to normal (group

III). When hamsters in groups IV and I were compared, there was no discernible change in the amounts of liver phase I and II functional agents (Supplementary Figure 2).

Effects of lycorine on TBARS and antioxidant enzymes in plasma

The levels of LPO byproduct TBARS were augmented while SOD, CAT, GPx, GSH, and vitamin E were decreased in the plasma of the DMBA-induced group. The level of TBARS was greatly reduced, and antioxidant levels were significantly enhanced to the normal level in DMBA-induced and lycorine-treated hamsters. The levels of TBARS and antioxidants were found to be normal when lycorine was given alone (Figure 2).

Effects of lycorine on TBARS and antioxidant enzymes in buccal mucosa

In contrast to the normal control hamsters, the status of TBARS, SOD, and CAT was attenuated, whereas the activities of GPx, GSH, and vitamin E were elevated in tumor-induced hamsters. The level of TBARS, SOD, and CAT were increased to near normal in lycorine (20 mg/kg b.w.) administrated and DMBA-induced hamsters. However, GPx, GSH, and vitamin E levels were lowered to near-normal levels. When lycorine (20 mg/kg b.w.) was administered alone, the TBARS and antioxidants levels were found to be normal (Supplementary Figure 3).

Effects of lycorine on p53, Caspase 3, and Cox-2 expression

Western blot analysis was used to look at the expression of the apoptotic markers p53, Caspase 3, and Cox-2 in the cheek pouches of all hamsters. The expression of p53 and Cox-2 was dramatically upregulated in DMBA-induced
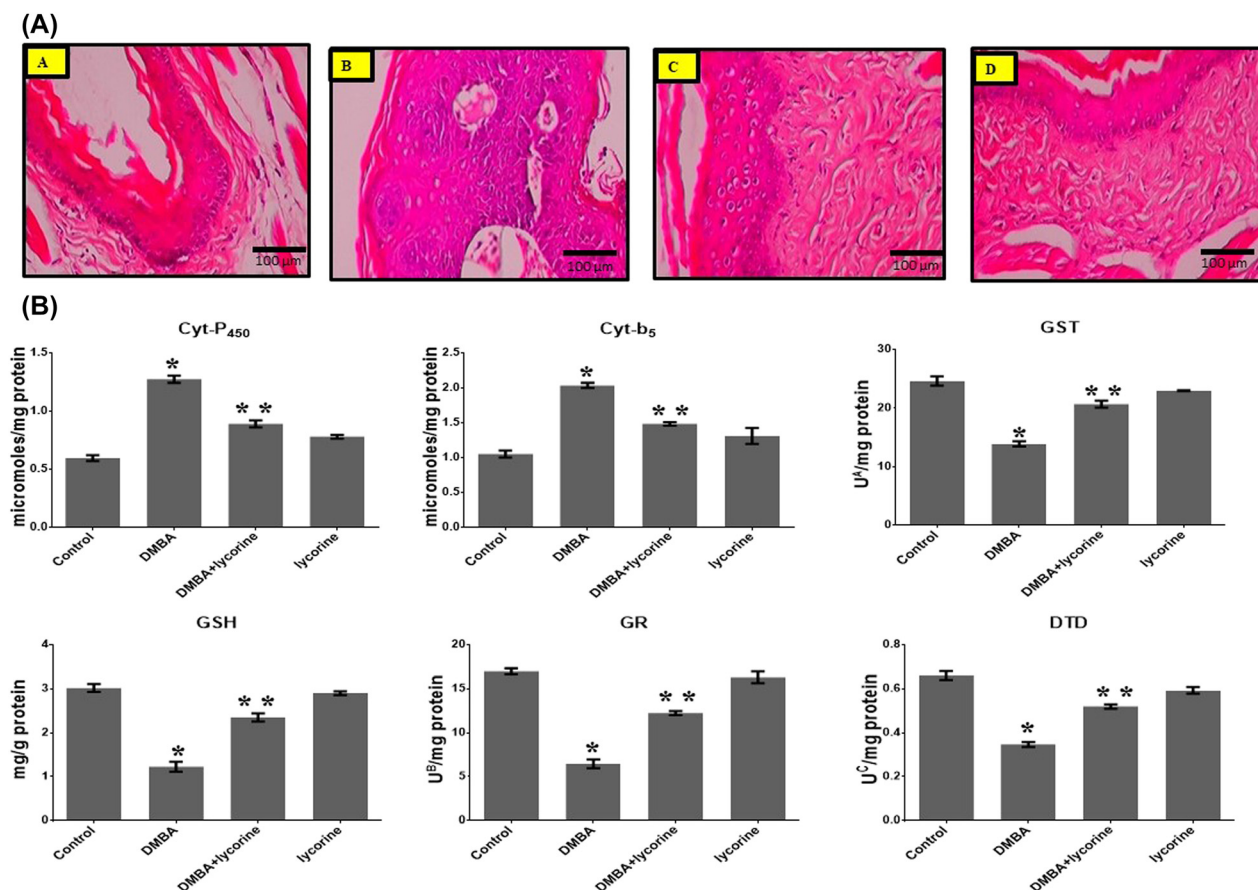
(A)

(B)

Figure 1: (A) Histopathological alterations in the buccal mucosa of all experimental hamsters. A and D images show distinct epithelium of buccal pouch in hamsters of normal control and lycorine-alone treated group. B images show well-differentiated squamous cell carcinoma DMBA alone treated animals. C images show very low or moderate dysplastic epithelium in hamsters treated with DMBA and lycorine. Images are showed in 40× magnification. (B) Effect of lycorine on the activities of phase I and phase II detoxification enzymes in the liver of all experimental hamsters. A-Micromoles of CDNB/GST conjugate developed per minute. B-Micromoles of NADPH oxidized per minute. C-Micromoles of 2,6-dichlorophenol reduced per minute. Values are shown as mean $\pm$ SD (n=6). *p<0.05, **p<0.01 vs. control group.

hamsters, however, caspase nine expressions were significantly downregulated. In DMBA-induced hamsters, lycorine treatment dramatically reduced p53 and Cox-2 expression, increasing caspase nine expressions to near-normal levels. Furthermore, lycorine treatment alone had no significant effect on the expression of p53, Cox-2, and caspases 9 (Figure 3A, B).

Effects of lycorine on PI3K/Akt expression

In DMBA-induced hamsters, P13K expression and phosphorylation of Akt are significantly increased than in control animals, according to western blot analysis; however, oral lycorine administration reduced P13K expression and phosphorylation of Akt in DMBA painted mice. In hamsters given lycorine (20 mg/kg b.w.) alone, no

significant differences in P13K/Akt expression were identified compared to control hamsters (Figure 3C, D).

Effect of lycorine on phosphorylation levels of Iκ-Bα and NF-κB

The results explore that the modulation of p-IκB-α and p-NF-κB were significantly upregulated in hamsters who received the painting of DMBA; however, it was reduced near to normal level in DMBA exposed, and lycorine received hamsters. Whereas the expression level of IκB-α was notably downregulated in DMBA alone received hamsters; however, the expression was upregulated almost equal to the normal level in the DMBA painted and lycorine (20 mg/kg b.w.) received hamsters. Remarkably, no changes in the modulation of p-IκB-α, p-NF-κB, and
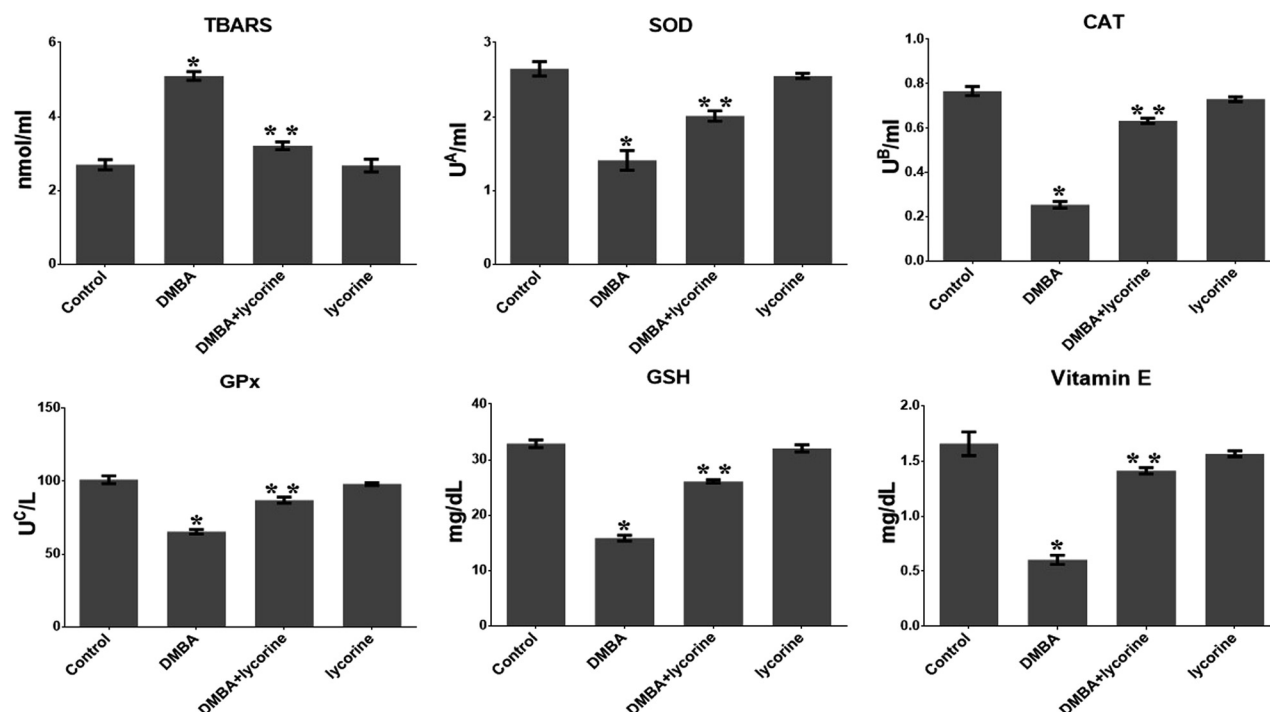
Figure 2: Effect of lycorine on TBARS and antioxidants in plasma of all experimental hamsters. (A) The volume of enzyme needed to inhibit 50% NBT reduction, (B) Micromoles of hydrogen peroxide utilized/second, (C) Micromoles of glutathione utilized/minute. Values are shown as mean ± SD (n=6). *$p<0.05$, **$p<0.01$ vs. control group.

IκBα has observed in hamsters that received lycorine alone (Figure 3E, F).

Discussion

Oral cancer has evolved into a significant clinical issue. However, a viable therapeutic and preventive plan for the various phases is required for the efficient treatment of oral cancer. Since 1954, oral cancer investigations have used hamster buccal pouch carcinogenesis [26]. The tumor-suppressing effect of lycorine in oral carcinogenesis in hamsters was studied in this work. In DMBA-painted hamsters, 100% tumor development was found. The oral lycorine therapy effectively reduced the tumor formation, tumor size, and histopathological alterations severity in tumor-induced hamsters. As a result, the current findings imply that lycorine has suppressed the aberrant tumor growth during oral tumorigenesis induced by DMBA. Even though lycorine has been known for its antioxidant, anti-cancer, and antimicrobial effects. There is no evidence in the literature that lycorine has an anti-carcinogenic action in DMBA-induced oral tumorigenesis. Liu et al. [27], found that lycorine inhibited the tumor cells, and promoted mitochondrial-dependent cell death in cancerous liver

cells through activating ROCK1. According to Zeng et al. [28], lycorine increased lung cancer cell death by modulating the AMPK-mTOR-S6K signaling pathway.

Hyperplasia, dysplasia is the hallmark of squamous cell cancer. The buccal mucosa of the hamsters was investigated for abnormal changes. We noticed significant hyperplasia, dysplasia, and well-differentiated squamous cell carcinoma in DMBA-induced hamsters. Although, in the DMBA-painted and lycorine (20 mg/kg b.w.) treated rats, the severity of hyperplasia, dysplasia, and squamous cell carcinoma in the buccal mucosa was reduced. The epithelial layers of hamsters given lycorine (20 mg/kg b.w.) alone were well defined and unchanged, similar to control hamsters. Similarly, the treatment of paeonol on DMBA-induced oral carcinogenic hamsters showed significant suppression of hyperplasia and dysplasia development [29].

Phase I enzymes are complicated in the stimulation of carcinogenic compounds, whereas phase II enzymes are complicated in the detoxification of carcinogenic chemicals [30]. The levels of phase I enzymes were found to be higher in the livers of DMA-induced hamsters, but phase II detoxifying indicators were lower. According to the current findings, the carcinogenic metabolite dihydrodiol epoxide was formed in excess during the metabolic stimulation of
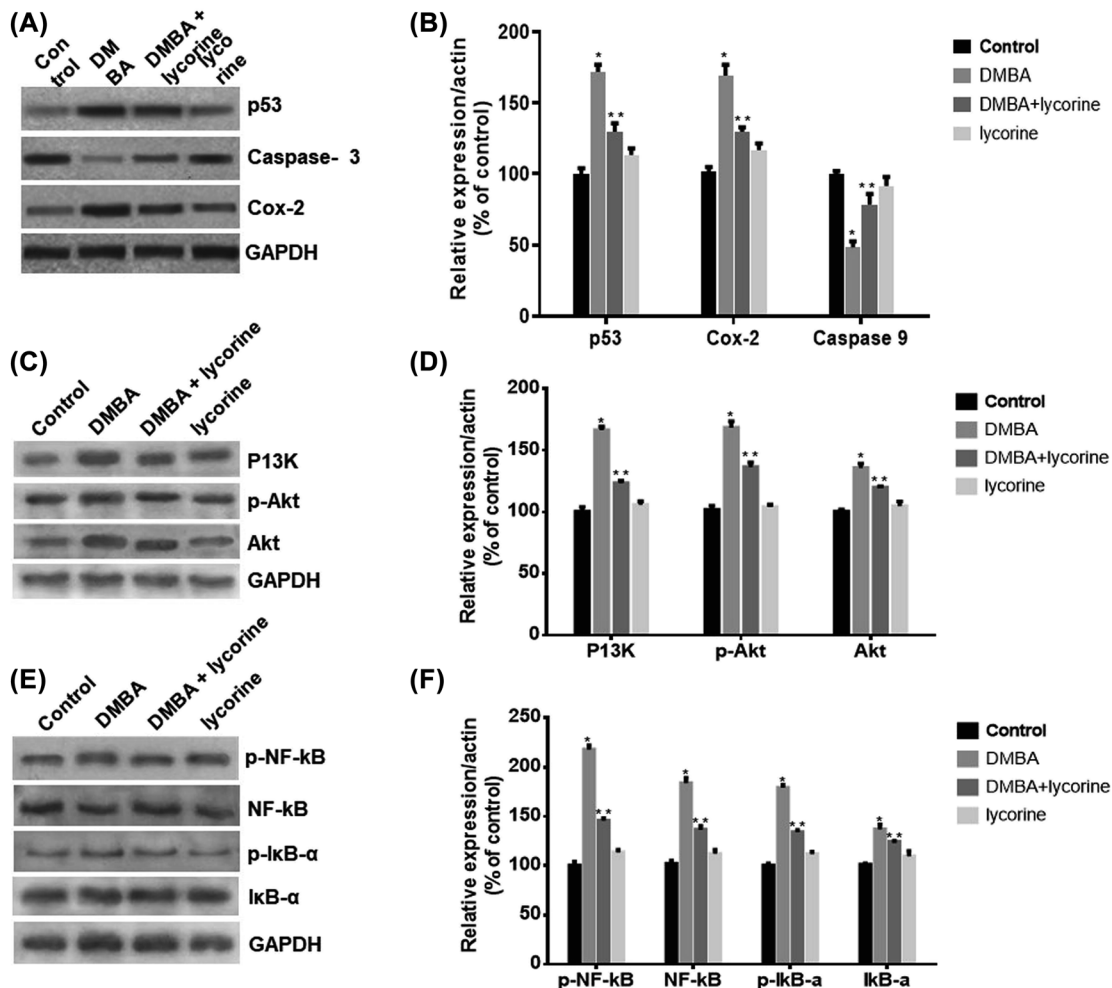
Figure 3: Effect of lycorine on apoptotic proteins p53, Caspase 9, Cox-2, P13K/Akt and NF-κB modulation by western blot investigation. (A) The oral administration of lycorine reduced the p53 and Cox-2 and increased the caspase nine expression cheek pouches tissue of DMBA exposed experimental hamsters. (B) The graphical representation shows the relative expression of p53, Caspase 9, Cox-2 vs GAPDH. (C) The oral administration of lycorine reduced the P13K expression and inhibited the phosphorylation of Akt in the cheek pouches tissue of DMBA exposed experimental hamsters. (D) The graphical representation shows the relative expression of P13K, p-AKT/AKT vs GAPDH. (E) The oral administration of lycorine reduced the phosphorylated-IκB-α and phosphorylated-NF-κB expression in the cheek pouches tissue of DMBA exposed experimental hamsters. (F) The graphical representation shows the relative expression of p-IκB-α/IκB-α, NF-κB/NF-κB vs. GAPDH. Values are shown as mean ± SD (n=6). *$p < 0.05$, **$p < 0.01$ vs. control group.

DMBA, limiting the levels of phase II detoxifying agents. Phase I and II detoxicants were dramatically elevated in hamsters induced with DMBA. Increased activity of phase I and II enzymes has been linked to frequent carcinogenic exposure in the buccal mucosa in several studies [31]. Our findings show that oral lycorine administration to DMBA-exposed hamsters reverted phase I and phase II enzyme levels to near-normal levels. The current data suggest that during DMBA-induced hamster oral carcinogenesis, lycorine altered phase I and II enzyme activity in favor of carcinogenic metabolite excretion.

We discovered elevated plasma TBARS and attenuated antioxidants in hamsters exposed to DMBA alone, confirming oxidative stress in DMAB-induced hamsters. Non-enzymatic and enzymatic antioxidant levels are likely to be lower due to their use by tumor tissues or to battle the damaging properties of LPO, respectively [32]. In the tumor tissue of DMBA-induced hamsters, the LPO was reduced, and antioxidants were disrupted compared to normal control hamsters. Low polyunsaturated fatty acid content and aberrant cell proliferation in tumor tissues have been proposed as causes of lower amounts of LPO. GSH and GPx activity increased in tumor tissues, most likely due to their controlling properties on cell proliferation. SOD and CAT activity reduction have been associated with various malignancies, including oral cancer [33]. After exposure to

lycorine, the activities of LPO and antioxidants in the plasma and buccal mucosa of DMBA-induced hamsters changed. Lycorine may have aided in maintaining the oxidant and antioxidant balance during oral carcinogenesis models. Thus, lycorine's anti-tumorigenesis effect in DMBA-induced oral tumor hamsters was revealed in this investigation.

The p53 mutation has been reported as an early event in the formation of OSCC, and its detection has been used as a biomarker for the early stages of carcinogenesis. Caspase-9 expression is associated with the development of tumors on the mouth floor. Caspase-9 downregulation has also been discovered during oral carcinogenesis [34]. COX-2 is a novel target for cancer prevention [35]. Cox-2 is expressed at modest levels in normal tissues, while it is highly expressed in cancer cells [36]. Our findings confirmed that the elevated expression of p53 and Cox-2 and down-regulated expression of Caspase nine during DMBA caused oral carcinogenesis. However, the lycorine treatment effectively reverted the expression level near to normal.

The PI3K/Akt signaling cascade is the most commonly impaired in cancer [37]. Most of the studies conducted on animal models found an increase in Akt protein expression from 50 to 70% due to activation of PI3K/Akt signaling. Receptor tyrosine kinases activate PI3K, and active PI3K activates Akt further. Akt activation then phosphorylates downstream PDK1 and mTOR molecules, activating transcription factors complicated in cell survival, growth, and proliferation [38]. In the current study, lycorine effectively reduced the progression of buccal pouch carcinoma by downregulating the expression of the PI3K/Akt signaling pathway, which confirms that lycorine is a potent anti-tumorigenic agent to treat the buccal pouch oral carcinoma. NF-κB, an essential transcription factor that is carried into the cell nucleus, controls various gene transcriptions involved in immunological and inflammatory responses, cell differentiation, and death. When NF-κB united with IκB in the cytoplasm, it lost its capability to control transcription. Once IκB was phosphorylated and liberated from the IκB/NF-κB complex, NF-κB regained this capacity [39]. We discovered that lycorine suppressed the nuclear import of NF-κB in DMBA-induced pouch oral cancer by lowering IκB and NF-κB phosphorylation. Previously, it was found that lycorine inhibits NF-κB activation and the production of inflammation-related cyclooxygenase at low concentrations. This finding implies that lycorine suppressed the NF-κB signaling pathway in DMBA-treated pouch oral tumor mice when given orally.

Conclusions

The present research work indicates lycorine has chemopreventive ability in DMBA-induced hamster buccal pouch carcinogenesis. During DMBA-induced tumorigenesis, the chemopreventive efficacy of lycorine is likely related to its modifying influence on phase I and II enzymes. Although hamsters given DMBA + lycorine did not develop tumors, substantial hyperplasia, and dysplasia. Moreover, the oral treatment of lycorine shows chemoprotective nature on DMBA-induced pouch oral carcinoma by downregulating the modulation of p53, Coc-2, P13K/Akt, and NF-κB signaling pathway. Therefore, using lycorine as an alternative to the available drugs for oral cancer treatment could improve oral cancer chemotherapeutic strategies.

References

1. Warnakulasuriya S. Living with oral cancer: epidemiology with particular reference to prevalence and life-style changes chemopreventive that influence survival. Oral Oncol 2010;46:407–10.
2. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol 2009;45:309–16.
3. Velu P, Vinothkumar V, Babukumar S, Ramachandhiran D. Chemopreventive effect of syringic acid on 7,12-dimethylbenz(a) anthracene induced hamster buccal pouch carcinogenesis. Toxicol Mech Methods 2017;27:631–40.
4. Mcfadyen MC, Melvin WT, Murray GI. Cytochrome P450 enzymes: novel options for cancer therapeutics. Mol Cancer Ther 2004;3:363–71.
5. Townsend DM, Tew KD. The role of glutathione-S-transferase in anti-cancer drug resistance. Oncogene 2003;22:7369–75.
6. Vidjaya Letchoumy P, Chandra Mohan KV, Stegeman JJ, Gelboin HV, Hara Y, Nagini S. Pretreatment with black tea polyphenols modulates xenobiotic-metabolizing enzymes in an experimental oral carcinogenesis model. Oncol Res 2008;17:75–85.
7. Beyer RE, Segura-Aguilar J, Di Bernardo S, Cavazzoni M, Fato R, Fiorentini D, et al. The role of DT-diaphorase in the

maintenance of the reduced antioxidant form of coenzyme Q in membrane systems. Proc Natl Acad Sci USA 1996;93: 2528–32.

8. Shi X, Wang J, Lei Y, Cong C, Tan D, Zhou X. Research progress on the PI3K/AKT signaling pathway in gynecological cancer (review). Mol Med Rep 2019;19:4529–35.

9. Liu R, Chen Y, Liu G, Li C, Song Y, Cao Z, et al. PI3K/AKT pathway as a key link modulates the multidrug resistance of cancers. Cell Death Dis 2020;11:797.

10. Guzman ML, Neering SJ, Upchurch D, Grimes B, Howard DS, Rizzieri DA, et al. Nuclear factor-kappaB is constitutively activated in primitive human acute myelogenous leukemia cells. Blood 2001;98:2301–7.

11. Mitchell S, Vargas J, Hoffmann A. Signaling via the NFκB system. Wiley Interdiscip Rev Syst Biol Med 2016;8:227–41.

12. Lamoral-Theys D, Pottier L, Dufrasne F, Nève J, Dubois J, Kornienko A, et al. Natural polyphenols that display anticancer properties through inhibition of kinase activity. Curr Med Chem 2010;17:812–25.

13. Ying X, Huang A, Xing Y, Lan L, Yi Z, He P. Lycorine inhibits breast cancer growth and metastasis via inducing apoptosis and blocking Src/FAK-involved pathway. Sci China Life Sci 2017;60: 417–28.

14. Vinothkumar V, Manoharan S. Chemopreventive efficacy of geraniol against 7,12-dimethylbenz[a]anthracene-induced hamster buccal pouch carcinogenesis. Redox Rep 2011;16: 91–100.

15. Omura T, Sato R. The carbon monoxide binding pigment of liver. J Biol Chem 1964;239:2370–8.

16. Habig WM, Pabst MJ, Jakoby WB. Glutathione S-transferase. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974;249:7130–9.

17. Carlberg I, Mannervik B. Glutathione reductase. In: Meister A, editor. Methods in enzymology. New York: Academic Press; 1985, vol 113:484–90 pp.

18. Ernster L. DT-diaphorase. Methods Enzymol 1967;10:309–17.

19. Yagi K. Lipid peroxides and human diseases. Chem Phys Lipids 1987;45:337–51.

20. Ohkawa H, Ohisi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95: 351–8.

21. Kakkar P, Das B, Visvanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys 1984; 21:130–2.

22. Sinha KA. Colorimetric assay of catalase. Anal Biochem 1972;17: 389–94.

23. Rotruck JT, Pope AL, Ganther HT, Swanson AB, Hafeman DG, Hockstra WG. Selenium: biochemical role as a component of glutathione peroxidase. Science 1973;179:588–90.

24. Paglia DE, Valentin WN. Studies on quantitative and qualitative characterization of erythrocyte gluatathione peroxidase. J Lab Clin Med 1967;95:351–8.

25. Desai ID. Vitamin E analysis, methods for animal tissues. Methods Enzymol 1984;105:138–45.

26. Palan PR, Mikhail BS, Basu J, Romney SL. Plasma levels of antioxidant betacarotene and alpha-tocopherol in uterine cervix dysplasias and cancer. Nutr Cancer 1991;15:13–20.

27. Liu WY, Tang Q, Zhang Q, Hu CP, Huang JB, Sheng FF, et al. Lycorine induces mitochondria-dependent apoptosis in hepatoblastoma HepG2 cells through ROCK1 activation. Front Pharmacol 2019;10:651.

28. Zeng H, Fu R, Yan L, Huang J. Lycorine induces apoptosis of A549 cells via AMPK-Mammalian Target of Rapamycin (mTOR)-S6K signaling pathway. Med Sci Mon Int Med J Exp Clin Res: Int Med J Exp Clin Res 2017;23:2035–41.

29. Ramachandhiran D, Vinothkumar V, Babukumar S. Paeonol exhibits anti-tumor effects by apoptotic and anti-inflammatory activities in 7,12-dimethylbenz(a)anthracene induced oral carcinogenesis. Biotech Histochem 2019;94:10–25.

30. Manoharan S, Balakrishnan S, Menon VP, Alias LM, Reena AR. Chemopreventive efficacy of curcumin and piperine during 7,12-dimethylbenz[a]anthracene-induced hamster buccal pouch carcinogenesis. Singap Med J 2009;50:139–46.

31. McLellan LI, Wolf CR. Glutathione and glutathione-dependent enzymes in cancer drug resistance. Drug Resist Updates 1999;2: 153–64.

32. Balasenthil S, Arivazhagan S, Nagini S. Garlic enhances circulatory antioxidants during 7,12 dimethylbenz(a)anthracene induced hamster buccal pouch carcinogenesis. J Ethnopharmacol 2000;72:429–33.

33. Gonzales R, Auclair C, Voisin E. Superoxide dismutase, catalase and glutathione peroxidase in red blood cells from patients with malignant diseases. Cancer Res 1984;44:4137–9.

34. Casto BC, Knobloch TJ, Galioto RL, Yu Z, Accurso BT, Warner BM. Chemoprevention of oral cancer by lyophilized strawberries. Anticancer Res 2013;33:4757–66.

35. Sarkar FH, Adsule S, Li Y, Padhye S. Back to the future: COX-2 inhibitors for chemoprevention and cancer therapy. Mini Rev Med Chem 2007;7:599–608.

36. Liu X, Ji Q, Ye N, Sui H, Zhou L, Zhu H, et al. Berberine inhibits invasion and metastasis of color- ectal cancer cells via COX-2/ PGE2 mediated JAK2/STAT3 signaling pathway. PLoS One 2015; 10:0123478.

37. Patnaik A, Appleman LJ, Mountz JM, Ramanathan RK, Beeram M, Tolcher AW. A first-in-human phase I study of intravenous PI3K inhibitor BAY 80-6946 in patients with advanced solid tumors: results of dose-escalation phase. J Clin Oncol 2011;29:3035.

38. Liu J, Chen W, Zhang H, Liu T, Zhao L. miR-214 targets the PTEN-mediated PI3K/Akt signaling pathway and regulates cell prolif- eration and apoptosis in ovarian cancer. Oncol Lett 2017;14: 5711–8.

39. Escárcega RO, Fuentes-Alexandro S, García-Carrasco M, Gatica A, Zamora A. The transcription factor nuclear factor-kappa B and cancer. Clin Oncol 2007;19:154–61.