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Syringic acid, a polyphenol modulates detoxification agents on 7,12-dimethylbenz[a]-anthracene induced oral carcinogenesis in golden Syrian hamsters

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Abstract
The status of detoxification agents were used as biochemical end points to assess the chemo preventive potential of syringic acid (SA), a polyphenol, in 7,12-dimethylbenz[a]- anthracene (DMBA)-induced hamster buccal pouch carcinogenesis. Topical application of 0.5% DMBA in liquid paraffin, thrice a week for 10 weeks on their buccal pouches hasmters. 100% tumor formation was noticed in hamsters treated with DMBA alone, orally administration of SA, at a dose of 50 mg/kg bw., to DMBA-treated hamster completely prevented the development of oral tumors. Moreover, SA significantly restored the status of phase I and phase II detoxification agents and favoring the secretion of carcinogenic metabolite, during DMBA-induced oral carcinogenesis. The present study concludes that the chemo preventive potential of SA relies on its modulatory effects on phase I and II detoxification agents to excrete the carcinogenic metabolite, during DMBA-induced hamster OSCC.

Keywords: syringic acid, polyphenol

Introduction
Cancer development is associated with increased cell proliferation formed in squamous cell carcinoma of the oral cavity (Baillie *et al*., 2017) [2]. The most common risk factors for oral carcinogenesis are tobacco smoking, betel quid chewing, alcohol drinking and viral infection (Stefano *et al*., 2013). On catabolism of DMBA, a procarcinogenic substance converted to ultimate carcinogenic form 7,12-DMBA-3,4-dihydrodiol-1,2-epoxied that causes tissue redox imbalance and excess generation of ROS, which results in oxidative damage in DNA bases forming adducts, damage cell membranes, lipids, proteins and nucleic acid leads to pathological outcome which results in carcinogenesis (Shimada *et al*., 2013; Ye *et al*., 2016) [22, 29]. Also, defect in detoxification cascade leads to accumulation of toxic metabolites, which causes mutation in DNA and leads to neoplastic transformation (Birben, 2012; Bhattacharyya *et al*., 2014) [5, 4]. Liver is the primary site for biotransformation of xenobiotics and detoxification measures. Phases I and II detoxification agents perform a crucial function in the metabolic activation and excretion of carcinogenic metabolites (Cederbaum, 2015) [8].
The hamster buccal pouch (HBP) carcinogenesis model is the best known animal system for investigating the efficacy of chemopreventive agents in oral carcinogenesis. Chemoprevention by dietary agents is a cost-effective method for the preventing of oral cancer incidence (Shklar and Oh, 2000) [23]. We therefore undertook the present study to investigate the chemopreventive potential of SA on DMBA-induced buccal pouch carcinogenesis in Syrian hamsters. The status of phase I and phase II enzymes were used as biomarkers of chemoprevention.
Oxidative stress defined as a state of imbalance between the concentrations of ROS and the antioxidant defense mechanisms. It is one of the most important phenotypes implicated in the conversion to normal cell into malignant one. Imbalance in oxidant and antioxidant status is one of the commonly observed phenomenons in several pathological conditions including cancer. The chemopreventive agent exhibited significant protective effects against various free radicals generated from pathological conditions including cancer (Guimaraes *et al*., 2007) [11].
The present study has investigated the chemopreventive effect of SA by utilizing the status of phase I and II detoxification enzymes as biochemical end points in DMBA-induced hamster buccal pouch carcinogenesis.

Materials and methods
Chemicals and reagents
SA (≥ 95% Purity, CAS NO: 530-57-4) and DMBA (≥ 95% Purity, CAS NO: 57-97-8) were purchased from Sigma Aldrich Chemicals Pvt. Ltd (Bangalore, Karnataka, India). All other chemicals used were of analytical grade, purchased from Hi media Laboratories Pvt. Ltd., Mumbai, India.

Animals
Male golden Syrian hamsters (*Mesocricetus auratus*), 8-10 weeks old, weighing 80-120 g, were purchased from the National Institute of Nutrition, Hyderabad, India and were maintained in the central animal house, Rajah Muthaiah medical college and hospital, Annamalai University, Annamalainagar, India. They were housed 8 propylene cages, each cage contains 5 hamsters were maintained separately and provided standard pellet diet (Amrut laboratory Animal Feed Mysore Feeds Limited, Bangalore, Karnataka, 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. The local institutional

animal ethics committee (Registration number 160/1999/CPCSEA) of Annamalai University approved the experimental design (Proposal number 1113; Dated 16.04.2015).

Preparation of SA and their oral administration

SA (fig 1) dissolved in 0.9% saline and oral supplementation animals three times a week for 14 weeks on days alternate to DMBA application, starting a week before the exposure to the carcinogen.

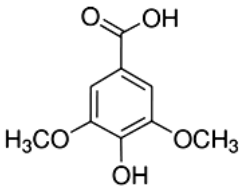

Fig 1: Chemical structure of SA

DMBA preparation and their topical application

DMBA (fig 2) 0.5% in liquid paraffin, painted on the left buccal pouches using a number 4 brush, three times a week for 10 weeks (Shklar, 1999) [24].

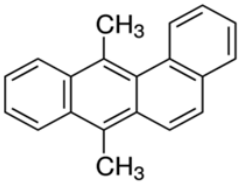

Fig 2: Structure of DMBA

Tumor assessment

The total number of tumors in each HBP was examined microscopically when the hamsters were euthanized and the diameter of each tumor was measured with a vernier caliper. Tumor volume was measured using the formula $V=\frac{4}{3}\pi\left(\frac{D_1}{2}\right)\left(\frac{D_2}{2}\right)\left(\frac{D_3}{2}\right)$, where D_1, D_2 and D_3 are the three diameters (mm^3) of the tumor. The tumor burden was calculated by multiplying the tumor volume and the number of tumors per hamsters.

Experimental design: (optimum dose study)

A total of 40 hamsters were randomized into four groups of 10 hamsters in each group. As shown in fig 3.

Group 1: Untreated untreated control hamsters

Group 2: DMBA (0.5% DMBA painted three times a week for 10 weeks).

Group 3: DMBA (0.5% DMBA painted three times a week for 10 weeks) + SA (50 mg/kg bw, by orally for 14 weeks on days alternate to DMBA painting).

Group 4: SA alone (50 mg/kg bw, by orally for 14 weeks on days alternate).

The experiment was terminated at the end of 14th weeks and all the hamsters were sacrificed by cervical dislocation. Animal body weight was calculated by subtracting the initial and final. Tissues for histopathological examination were immediately fixed in 10% neutral buffered formalin, embedded in paraffin, processed by histological technique stained with hematoxylin and eosin (H&E).

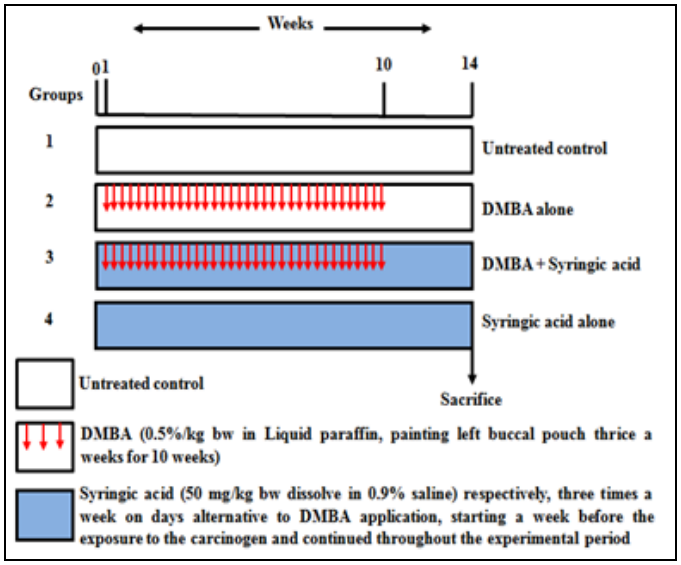

Fig 3: Experimental protocol

Biochemical analysis

Sample collection

Biochemical studies were conducted on liver and buccal mucosa of control and experimental hamsters in each group. Blood samples were collected into heparinized tubes. Tissue samples were washed with ice cold saline and homogenized using the appropriate buffer in an All-glass homogenizer with a Teflon (DuPont, Wilmington, Delaware, USA) pestle and used for biochemical estimations.

The protein content was estimated by the method of Lowry *et al*., (1951) [17]. The level of detoxification metabolizing agents such as cytochrome-p450 (cyt-p450) and cytochrome-b5 (cyt-b5), DT-diaphorase (DTD), glutathione-S-transferase (GST), glutathione reductase (GR), reduced glutathione (GSH) and oxidised glutathione (GSSG) liver and buccal mucosal tissue according to the methods of Omura and Sato (1964) [18], Lind *et al*. (1990) [16], Habig *et al*. (1974) [12], Carlberg and Mannervik (1985) [7] and Anderson (1985) [1], Tietze, (1969) [26] and Ernster, (1967) respectively [10].

Results

Body weight

Table 1 shows the initial and final body weight of control and experimental hamsters in each group. The mean body weight (89.60) was significantly ($p < 0.05$) decreased in DMBA alone painted hamsters as compared to untreated control hamsters. However, oral administration of SA (50 mg/kg bw), three times per week for 14 weeks significantly ($p < 0.05$) increased body weight (125.42) in DMBA treated hamsters. Oral administration of SA alone treated hamsters showed no significant difference in body weight (139.67) as compared to untreated control hamsters.

Table 1: Initial and final body weight of control and experimental hamsters in each group (n=10).

Groups/Treatment*	Initial body weight	Final body weight
Untreated control	115.11 ± 8.77[a]	137.02 ± 10.43[a]
DMBA	121.65 ± 9.26[a]	89.60 ± 6.82[b]
DMBA + SA (50 mg/kg bw)	113.44 ± 8.68[a]	125.42 ± 9.86[c]
SA-Alone (50 mg/kg bw)	114.31 ± 8.70[a]	139.67 ± 10.67[a]

*The hamsters left buccal pouch with topical application of 0.5% DMBA thrice a week for 10 weeks. Oral pre-administration of SA to group 3-4 hamsters for 14 consecutive weeks on days alternate to DMBA painting. Values are expressed as mean ± SD for ten hamsters in each group. Values not sharing a common superscript letter differ significantly at [(a-c)] $p < 0.05$.

Tumor incidence, tumor volume, tumor number and tumor burden

Table 2 shows the tumor incidence, tumor volume, tumor number and tumor burden of control and experimental hamsters in each group. In DMBA alone painted hamsters a 100% tumor formation with mean tumor volume (215.72) and tumor burden (2156.88) was observed. Oral administration of SA (50 mg/kg bw) completely prevented tumor incidence in DMBA painted hamsters. No tumor was observed in untreated control as well as SA alone treated hamsters.

Table 2: Tumor incidence, number, volume and burden of control and experimental hamsters in each group (n = 10).

Groups/ Treatment	Untreated control	DMBA	DMBA + SA (50 mg/kg bw)	SA-Alone (50 mg/kg bw)
Tumor incidence	0%	100%	0%	0%
Total number of tumor/hamsters	0	10 ± 0.76	0	0
Tumor volume (mm^3)/hamsters	0	215.72 ± 16.43	0	0
Tumor burden (mm^3)/hamsters	0	2156.88 ± 65.39	0	0

Values are expressed as mean ± SD for ten hamsters in each group. Values not sharing a common superscript letter differ significantly at $p < 0.05$. The total number of tumors in each HBP was examined microscopically when the hamsters were euthanized and the diameter of each tumor was measured with a vernier caliper. Tumor volume was measured using the formula $V=\frac{4}{3}\pi\left(\frac{D_1}{2}\right)\left(\frac{D_2}{2}\right)\left(\frac{D_3}{2}\right)$, where D_1, D_2 and D_3 are the three diameters (mm^3) of the tumor. The tumor burden was calculated by multiplying the tumor volume and the number of tumors per hamsters. Values are expressed as mean ± SD for ten hamsters in each group.

Histopathological observations

The histopathological differences were observed in the buccal mucosa of control and experimental hamsters in each group (fig 4 and Table 3). The histopathological analysis on untreated control hamsters and SA alone showed normal buccal mucosal epithelium (fig 4A and D). The buccal tissues from the DMBA alone painted hamsters depicted that severe keratosis, hyperplasia, dysplasia and well differentiated exophytic squamous cell carcinoma with keratin pearls, hyperchromatic modulations were appeared fig 4B. The buccal mucosa from the oral administered SA (50 mg/kg bw) to DMBA treated hamsters showed mild to moderate keratosis and mild hyperplasia (fig 4C).

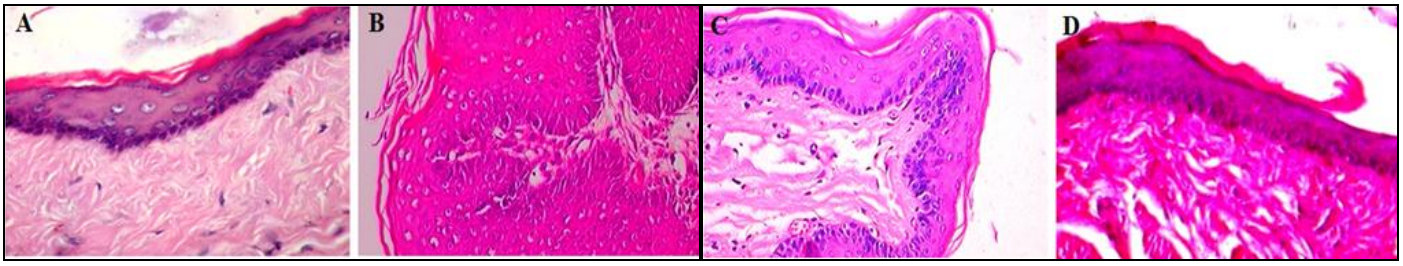

Fig 4: Photomicrograph showing the histopathological changes in the buccal mucosa of untreated control and experimental hamsters (H&E, 40x) in each group (n = 10).

Group 1 and 4 hamster buccal pouches showing well defined and intact epithelial layers. Group 2 showing well differentiated squamous cell carcinoma with keratin pearls in hamsters treated with DMBA alone. Group 3 showing mild to moderate keratosis and mild hyperplastic epithelium in hamsters treated with SA 50 mg/kg bw.

Effect of SA on phase I and II detoxification agents in the liver

Table 4 shows the status of phase I (Cyt P450 and b5) and phase II (GSH, GST, GR and DTD) detoxification enzymes, in the liver of control and experimental hamsters in each group. The status of phase I enzymes were enhanced, whereas phase II enzymes were significantly ($p < 0.05$) diminished in hamsters treated with DMBA alone (group 2) as compared with untreated control hamsters. Oral administration of SA at 50 mg/kg bw, with DMBA-treated hamsters (group 3) significantly ($p < 0.05$) brought back the status of phase I and II enzyme to near normal range. However, hamsters treated with SA alone 50 mg/kg bw, (group 4) showed no significant modification in phase I and II enzyme status as compared to untreated control hamsters (Group 1).

Table 4: The status of phase I and phase II detoxification enzymes in the liver of experimental hamsters in each group (n = 10).

Groups/Treatment	Untreated control	DMBA	DMBA + SA (50 mg/kg bw)	SA-Alone (50 mg/kg bw)
liver				
CYT P_{450} (U^x/mg protein)	0.69 ± 0.05^a	1.71 ± 0.13^b	0.95 ± 0.07^c	0.80 ± 0.05^a
CYT b_5 (U^y/mg protein)	1.26 ± 0.10^a	2.47 ± 0.19^b	1.41 ± 0.11^c	1.38 ± 0.10^a
Liver				
GSH (nmol/mg protein)	2.81 ± 0.21^a	0.90 ± 0.07^a	2.61 ± 0.20^c	2.95 ± 0.21^a
GST (U^A/mg protein)	37.27 ± 2.84^a	12.82 ± 0.98^a	31.90 ± 2.43^c	38.93 ± 2.88^a
GR (U^B/mg protein)	17.03 ± 1.30^a	8.53 ± 0.65^a	14.67 ± 1.12^c	16.06 ± 1.31^a
DTD (U^C/mg protein)	0.76 ± 0.06^a	0.31 ± 0.02^a	0.59 ± 0.04^c	0.80 ± 0.06^a

U^x – µmoles of cytochrome P_{450}, U^y – µmoles of cytochrome b_5, U^A – µmoles of 1-chloro-2, 4-dinitro benzene reduced glutathione conjugate formed/minute, U^B – µmoles of NADPH oxidized/ hour, U^C – µmoles of 2,6-dichloro indophenols reduced/ minute. Values are expressed as mean ± SD for six animals in each group. Values not sharing a common superscript ($^{a-c}$) differ significantly at $p<0.05$ (DMRT).

Effect of SA on phase I and II detoxification agents in the buccal mucosa

Table 5 shows the status of phase I (Cyt p450 and b5) and phase II (GST, GR, GSH, GSSG and GSH/GSSG) detoxification enzymes in the buccal mucosa of control and experimental hamsters. In the buccal mucosa, the activities of phase I (Cyt p450 and b5) detoxification agents where significantly ($p < 0.05$) increased, where as phase II altered (GST, GR, GSH, GSSG and GSH/GSSG ratio were increased; GSSG was decreased) in hamsters treated with DMBA alone (group 2) as compared with untreated control hamsters. Oral administration of SA at 50 mg/kg bw, to DMBA-treated hamsters (group 3) significantly ($p < 0.05$) restored the activities of phase I and II enzyme to near normal range. However, hamsters treated with SA alone (group 5) showed no significant difference in phase I and II enzyme levels as when compared to untreated control hamsters. The current study thus concluded that SA inhibited the metabolic activation of DMBA and enhanced elevation of carcinogenic metabolites through triggering the activities of phase I and II detoxification cascade in the buccal mucosa.

Table 5: The status of phase I and phase II detoxification enzymes in the buccal mucosa of experimental hamsters in each group (n = 10).

Groups/Treatment	Untreated control	DMBA	DMBA + SA (50 mg/kg bw)	SA-Alone (50 mg/kg bw)
Buccal mucosa				
CYT P_{450} (U^x/mg protein)	1.33 ± 0.10^a	3.26 ± 0.25^a	1.45 ± 0.11^c	1.42 ± 0.11^a
CYT b_5 (U^y/mg protein)	0.41 ± 0.03^a	0.69 ± 0.05^a	0.45 ± 0.03^c	0.49 ± 0.03^a
Buccal mucosa				
GST (U^A/mg protein)	1.45 ± 0.11^a	3.48 ± 0.27^a	1.61 ± 0.12^c	1.47 ± 0.12^a
GR (U^B/mg protein)	57.92 ± 4.41^a	119.16 ± 9.12^b	63.74 ± 4.88^c	59.21 ± 4.61^a
GSH (µg/g tissue)	59.39 ± 4.58^a	113.07 ± 8.61^a	64.02 ± 4.87^c	59.85 ± 4.69^a
GSSG (µg/g tissue)	13.71 ± 0.89^a	9.16 ± 0.70^a	12.03 ± 0.92^c	14.33 ± 1.02^a
GSH/ GSSG (µg/g protein)	4.33 ± 32^a	12.34 ± 0.94^a	5.32 ± 0.41^c	4.17 ± 0.35^a

U^x – µmoles of cytochrome P_{450}, U^y – µmoles of cytochrome b_5, U^A – µmoles of 1-chloro-2, 4-dinitro benzene reduced glutathione conjugate formed/minute, U^B – µmoles of NADPH oxidized/ hour, U^C – µmoles of 2,6-dichloro indophenols reduced/ minute. Values are expressed as mean ± SD for 10 hamsters in each group. Values not sharing a common superscript ($^{a-c}$) differ significantly at $p<0.05$ (DMRT).

Discussion

In the present study, the chemopreventive effect of SA was analyzed in DMBA induced hamsters buccal mucosal oral carcinogenesis, due to their various pharmacological actions on different cancers both *in vitro* and *in vivo* (Boutayeb and Boutayeb 2005) [6]. Current treatment methods against oral cancer are limited by the number of drugs and their side effects which warrant the need of new chemopreventive agents against oral cancer. Even though SA has been shown to possess anticancer property aganist many cancer models (Siew Hon Ng, 2011, Itoh *et al*., 2009, Ramachandran and Raja, 2010) [25, 13, 19]. There is no data available in the literature regarding its chemopreventive effect of SA on DMBA-induced oral cancer. Topical application of DMBA to the hamster buccal mucosa for 10 weeks, we observed decreased body weight, 100% tumor formation with very high tumor incidence, number, volume and burden, which was histopathologically confirmed as well as differentiated exophytic SCC. DMBA, a potent pro-carcinogen affects the hamsters, which is evidenced by a substantial levels of body weight decline. There also significant decrease in the food intake due to the occurrence of tumor in the buccal mucosa, resultant in reduced body weight in DMBA alone treated hamsters (Kamaraj *et al*., 2010) [14]. Earlier study documented that carcinogen increases the progressions of tumor leads to inhibition of growth rate of animals (Li *et al*., 2002) [15]. Our results corroborate with these finding. When treating with SA (50 mg/kg bw) in DMBA treated hamsters shows the increased body weight and completely prevented tumor number, volume and burden in the buccal mucosa when compared to DMBA and untreated control hamsters. A mild to moderated keratosis and a mild hyperplasia were depicted in the SA (50 mg/kg bw) treated hamsters, which was probably

due to the mechanical irritation of the brush for 10 weeks. From this results, we suggested that SA (50 mg/kg bw) orally administered significantly delayed the tumor formation. In our previous study reported that SA (50 and 100 mg/kg bw) exerts chemopreventive effect to restored the activity of biochemical and molecular markers against DMBA induced hamsters oral cancer (Velu *et al*., 2017) [27]. Yan *et al*. (2016) [28] demonstrated that mice administered with SA showed production against ethanol induced hepatotoxicity. Ramachandran and Raja, (2010) [19] demonstrated that SA (50 mg/kg bw) significantly decreased the activity of hepatic and renal markers against acetaminophen (APAP)-induced hepatotoxicity in rats.

Liver play major role in the metabolic homeostasis of toxic substances which are eliminated through detoxification agents and thus assessment of these agents could useful for the chemopreventive potential of SA during DMBA-induced buccal pouch carcinogenesis. DMBA undergoes metabolic activation forming reactive intermediates diol epoxide which is capable of binding covalently to adenine residues of DNA forming adducts that could ultimately leads to riding of normal cells into a malignant phenotype. Extensive study have documented that the activities of phase I detoxification enzymes (Cyt P450 and Cyt b5) were increased significantly in both liver and tumor tissues of hamsters treated with DMBA, due to oxidative reactions occurred with procarinognes/promutagens (Baskaran *et al*., 2017) [3]. Our data corroborate with above findings. GST, GR, DTD, GSSG and GSH are plays a major role in the detoxication of epoxides formed from aromatic hydrocarbons which include DMBA and these metabolic intermediates formed by phase I enzymes. DTD, a flavoprotein, helps the 2-electron reduction of the metabolic merchandise of polycyclic aromatic hydrocarbons inclusive of quinines, thereby stopping the formation of semiquinones (Danson *et al*., 2004) [9]. Disturbed activities of phase II agents are associated with many types of cancer (Sheweita and Tilmisany, 2003) [21]. In our present study, the activities of phase II detoxification agents were altered significantly in both liver and buccal mucosa in hamsters painted with DMBA, which represents that both liver and buccal tissue were affected by carcinogenic metabolites. Previous literature has shown that phenolic compounds are act as transcriptional activators of phase II xenobiotic enzymes. These agents could substantially stimulate antioxidant response elements (ARE) in the activated region of phase II detoxification genes and transcriptional factors are associated with excretion of carcinogens (Romilly *et al*., 2015) [20]. SA supplementation restored the status of xenobiotic agents in both liver and buccal tissue, which indicates that SA inhibited the metabolic activation and the elimination of carcinogenic metabolites during DMBA-induced hamster buccal pouch cancer.

Conclusion

Therefore, the present study concludes that SA has potent chemopreventive agent, due to its modulating effect on phase I and II xenobiotic metabolizing agents in favor of the excretion of carcinogenic metabolites during DMBA-induced oral carcinogenesis

References

1. Anderson ME. Determination of glutathione and glutathione disulfide in biological samples. Methods in Enzymology, 1985l; 113:548-555.
2. Baillie R, Tan ST, Itinteang T. Cancer Stem Cells in Oral Cavity Squamous Cell Carcinoma: A Review. Frontiers in Oncology, 2017; 7:112.
3. Baskaran N, Selvam GS, Yuvaraj S, Abhishek A. Parthenolide attenuates 7,12-dimethylbenz[a]anthracene induced hamster buccal pouch carcinogenesis. Journal of molecular cell biochemistry, 2017; 12:3151-3155.
4. Bhattacharyya A, Chattopadhyay R, Mitra S, Sheila E. Oxidative Stress: An Essential Factor in the Pathogenesis of Gastrointestinal Mucosal Diseases. Physiological Reviews. 2014; 94(2):329-354.
5. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative Stress and Antioxidant Defense. World Allergy Organization Journal. 2012; 5(1):9-19.
6. Boutayeb A, Boutayeb ST. The burden of non-communicable diseases in developing countries. International Journal for Equity in Health, 2005; 4:2.
7. Carlberg I, Mannervik B. Glutathione reductase. Methods in Enzymology, 1985; 113:484-490.
8. Cederbaum I. Molecular mechanisms of the microsomal mixed function oxidases and biological and pathological implications. Redox Biology, 2015; 4:60-73.
9. Danson S, Ward TH, Butler J, Ranson M. DT-diaphorase: a target for new anticancer drugs. Cancer Treatment Reviews. 2004; 30(5):437-449.
10. Ernster L. DT-Diaphorase. In: Estabrook RW, Pullman ME eds. Methods in enzymology. New York: Academic Press, 1967; 10:309-317.
11. Guimaraes CM, Giao MS, Martinez SS, Pintado AI, Pintado ME, Bento LS *et al.* Antioxidant activity of sugar molasses, including protective effect against DNA oxidative damage. Journal of Food Science. 2007; 72(1):C039-43.
12. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferase. The first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry, 1974; 249:7130-7139.
13. Itoh A, Isoda K, Kondoh M, Kawase M, Watari A, Kobayashi M *et al.* Hepatoprotective effect of syringic acid and vanillic acid on CCl4-induced liver injury. Biological and Pharmaceutical Bulletin, 2009; 33:983-987.
14. Kamaraj S, Anandakumar P, Jagan S, Ramakrishnan G, Devaki T. Modulatory effect of hesperidin on benzo (a) pyrene induced experimental lung carcinogenesis with reference to COX-2, MMP-2 and MMP-9. European Journal of Pharmacology, 2010; 649:320-327.
15. Li N, Chen X, Liao J, Yang G, Wang S, Josephson Y *et al.* Inhibition of 7,12-dimethylbenz[a]anthracene DMBA-induced oral carcinogenesis in hamsters by tea and curcumin. Carcinogenesis. 2002; 23(8):1307-13.
16. Lind C, Cadenas E, Hochstein P, Ernster L. DT-diaphorase: purification, properties, and function. Methods in Enzymology, 1990; 186:287-301.

17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. Journal of Biological Chemistry, 1951; 93:265-275.
18. Omura T, Sato R. The carbon monoxide binding pigment of liver microsomes: phase I evidence for its hemoprotein nature. Journal of Biological Chemistry, 1964; 239:2370-2378.
19. Ramachandran V, Raja B. Protective effects of syringic acid against acetaminophen-induced hepatic damage in albino rats. Journal of Basic and Clinical Physiology and Pharmacology. 2010; 21(4):369-385.
20. Romilly E, Hodges, Deanna M Minich. Modulation of Metabolic Detoxification Pathways Using Foods and Food-Derived Components: A Scientific Review with Clinical Application. Journal of Nutrition and Metabolism, 2015; 2015:760689.
21. Sheweita SA, Tilmisany AK. Cancer and phase II drug-metabolizing enzymes. Current Drug Metabolism. 2003; 4(1):45-58.
22. Shimada T, Murayama N, Yamazaki H, Tanaka K, Takenaka S, Komori M *et al.* Metabolic Activation of Polycyclic Aromatic Hydrocarbons and Aryl and Heterocyclic Amines by Human Cytochromes P450 2A13 and 2A6. Chemical Research in Toxicology. 2013; 26(4):529-537.
23. Shklar G, Oh SK. Cancer Invest. Experimental basis for cancer prevention by vitamin E. Cancer Investigation. 2000; 18(3):214-222.
24. Shklar G. Development of experimental oral carcinogenesis and its impact on current oral cancer research. Journal of Dental Research, 1999; 78:1768-1772.
25. Siew Hon N. Master Thesis. Characterization colon cancer cell culture Based Screening Assay to study Effects of Phenolic Acids. University Saskatchewan, 2011, 1-173.
26. Tietze F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Analytical Biochemistry, 1969; 27:502-522.
27. Velu P, Vinothkumar V, Babukumar S, Ramachandhiran D. Chemopreventive effect of syringic acid on 7,12-dimethylbenz(a)anthracene induced hamster buccal pouch carcinogenesis. Toxicology Mechanisms and Methods. 2017; 27(8):631-640.
28. Yan SL, Wang ZH, Yen HF, Lee YJ, Yin MC. Reversal of ethanol-induced hepatotoxicity by cinnamic and syringic acids in mice. Food and Chemical Toxicology, 2016; 98:119-126.
29. Ye R, Shi M, Liu Q, Chen J. Redox Imbalance and Stroke. Oxidative Medicine and Cellular Longevity, 2016, 3065263.