ANTI-LIPIDPEROXIDATIVE AND ANTIOXIDANT EFFECTS OF *ZINGIBER OFFICINALE* ROSCOE ROOT EXTRACT IN 7, 12-DIMETHYL BENZ[A] ANTHRACENE INDUCED ORAL CARCINOGENESIS

K. Suresh*[1], K. Sivakumar[2], M.A. Vijayaanand[1], K. Rajalingam[1], G. Rajkamal[1]
Department of Biochemistry & Biotechnology[1], Department of Botany[2],
Annamalai University, Annamalai Nagar, Tamilnadu, India-608 002.

*Corresponding author
Phone: +91-4144-239141 (Extn. *209) (Off)
Fax: +91-4144-238080
E-Mail: suraj_cks@yahoo.co.in

Summary

The present study has investigated the chemopreventive and antilipidperoxidative efficacy of *Zingiber officinale* Roscoe aqueous root extract in 7,12-Dimethylbenz[a]anthracene (DMBA) induced hamster buccal pouch carcinogenesis. Oral squamous cell carcinoma was induced in hamster buccal pouches by painting with 0.5% 7, 12-Dimethylbenz[a]anthracene (DMBA) three times per week for 14 weeks. 100% tumor formation, altered levels of lipid peroxidation and antioxidants status were observed in DMBA painted hamsters. Oral administration of *Zingiber officinale* Roscoe aqueous root extract significantly prevented the tumor formation as well as decreased the levels of lipid peroxidation by products and enhanced the antioxidants defense mechanism in DMBA treated hamsters.

Key words: DMBA, Oral cancer, *Zingiber officinale*, Lipid peroxidation, Antioxidants.

Introduction

Oral squamous cell carcinoma (OSCC) of the oral cavity, the fifth most common cancer worldwide, is the leading cause of morbidity and mortality in the Indian subcontinent (1). In India, where the habits of chewing tobacco with betel nut, reverse smoking and heavy alcohol usage are common risk factors, there is a striking incidence of oral cancer which accounts for as many as 30-40% of all cancers (2). 7,12-Dimethylbenz(a)anthracene (DMBA) is commonly used potent organ and site-specific carcinogen to induce buccal pouch carcinogenesis in hamsters, The hamster oral cancer model has relevant similarities to human oral cancer development (3).

DMBA is metabolized to dihydro diol-epoxide, the ultimate carcinogen, which mediates carcinogenic process by inducing chronic inflammation, over production of reactive oxygen species (ROS) and oxidative DNA damage (4).

The most important free radicals in the reactive oxygen species (ROS) are singlet oxygen (O_2), super oxide anions ($O_2\cdot$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\cdot OH$) in the etiology of cancer (5). Free radicals induced oxidative stress has been implicated in the pathogenesis of several diseases including cancer. Free radicals can damage proteins, lipids, carbohydrates, and nucleic acids. The most important function of free radicals *in vivo* or *in vitro* is lipid peroxidation resulting in deleterious effects on membrane system and damages the cells (6).

Antioxidants are intimately involved in the prevention of cellular damage is the common pathway for cancer, aging and a variety of diseases (7). However, the body has developed several endogenous antioxidants defense systems (non enzymatic and enzymatic) to deal with the production of reactive oxygen intermediates. The antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (Gpx) and the non-enzymatic antioxidants include vitamin E, vitamin C and reduced glutathione (GSH) (8).

Ginger (*Zingiber officinale* Roscoe, Zingiberaceae) is widely used as a dietary condiment throughout the world. Besides its extensive utilization as a spice, the rhizome of ginger has been used traditional oriental medicine to ameliorate such symptoms as inflammation, rheumatic disorders and gastrointestinal discomforts (9). Ginger is used extensively in traditional Chinese medicine to treat headaches, nausea and colds and in Ayurvedic and western herbal medicinal practice for the treatment of arthritis, rheumatoid disorders and muscular discomforts (10). Ginger is often used for the treatment of stomachache, and cardiovascular and motor diseases. It also possesses anti-inflammatory activity and regulates bacterial growth, as well as providing protection for immune-depressed patients, such as individuals who are HIV positive (11). This species contains biologically active constituents including the main pungent principles, the gingerols and shogaols (12).

However, no scientific reports were available on the literature for its chemopreventive and anti-lipidperoxidative effects in DMBA induced buccal pouch carcinogenesis. In the present study, the chemopreventive and anti-lipidperoxidative effect of ginger was examined in DMBA induced experimental oral carcinogenesis.

Materials and Methods

Chemicals

The carcinogen, 7, 12-Dimethylbenz(a)anthracene (DMBA), was obtained from Sigma-Aldrich chemical Pvt. Ltd. Bangalore, India. All other chemicals used were of analytical grade.

Animals

Male golden Syrian hamsters, 8-10 weeks old, weighing 80-120g were purchased from National Institute of Nutrition, Hyderabad, India and maintained in central animal house, Rajah Muthaiah Medical College and Hospital, Annamalai University. The animals were housed in poly propylene cages and provided standard pellet and water *add libitum*. The animals were maintained under controlled conditions of temperature and humidity with a 12h light dark cycle.

Plant material

Zingiber officinale roots were purchased from fresh market in Chidambaram, Tamil nadu, India and authenticated by the Botanist, Dr.K.Sivakumar, Department of Botany, and Annamalai University. A voucher specimen (AU04219) was also deposited.

Preparation of the plant extracts

Five hundred grams of dried and finely powdered *Zingiber officinale* root were suspended in 250 ml of water for 2h and then heated at 60-65•**C** for 30 min. the extracts was preserved and the process was repeated for three times with the residual powder, each time collecting the extract was pooled and passed through the fine cotton cloth. The filtrates upon evaporation at 40•**C** yielded 16% semisolid extract. This was stored at 0-4•**C** until used.

A known volume of residual extracts was suspended in distilled water and was orally administered to the animals by gastric intubation using a force-feeding needle during the experimental period.

Experimental Protocol

The local Institutional animal ethics committee, Annamalai University, Annamalai Nagar, India, has approved the experimental design. A total number of 24 golden Syrian Hamsters were randomized into 6 animals in each. Group I animals were served as untreated control. Groups II animals were painted with 0.5% DMBA in liquid paraffin three times per week for 14 weeks on the left buccal pouches. Group III orally administered with *Zingiber officinale* aqueous root extracts (ZoARet) (500 mg kg-1 bw) starting 1 week before the exposure to the carcinogen and continued on days alternate to DMBA painting, until the scarification of the animals. Group IV received ZoARet (500 mg kg-1 B.wt) alone throughout the experimental period. The experiment was terminated at the end of 14^{th} week and all animals were sacrificed by cervical dislocation. Biochemical studies were conducted on blood and buccal mucosa of control and experimental animals in each group. For histopathological examination, buccal mucosa tissues were fixed in 10% formalin and routinely processed and embedded with paraffin, 2-3µm sections were cut in a rotary microtome stained with haematoxylin and eosin.

Biochemical analysis

After plasma separation, the buffy coat was removed and the packed cells were washed thrice with physiological saline. A known volume of erythrocytes was lysed with hypotonic buffer at pH 7.4. The heamolysate was separated by centrifugation at 10,000 rpm for 15 min at 20˚c. The erythrocyte membrane was prepared by the method of Dodge et al (13). Modified by Quis(14). Thiobarbituric acid reactive substances (TBARS) were assayed in plasma, erythrocytes, and buccal mucosa according to the methods of Yagi, Donnan and Okhawa etal (15, 16, 17) respectively. Reduced glutathione (GSH) was determined by the method of Beutler and Kelley (18). Vitamin C and E were measured according to the methods of Omaye et al (19) and Desai (20), respectively. The activities of enzymatic antioxidants, SOD, CAT and Gpx were estimated by the methods of Kakkar et al (21) Sinha (22) and Rotruck et al (23) respectively.

Statistical analysis

Values are expressed as mean ± SD. Statistical analysis was performed by One-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The Values were considered statistically significant if the p-value was less than 0.05.

Results

Table1 shows the effect of *Zingiber officinale* Roscoe root extracts on tumor incidence, tumor volume, and tumor burden and histopathological features in DMBA induced hamster buccal pouch carcinogenesis. We observed 100% tumor formation with mean tumor volume (70.37±55.9mm3) and tumor burden (140.64±7.88 mm3) in DMBA alone painted hamsters (Group II). Oral administration of ZoARet at a dose of 500 mg kg-1 body weight significantly prevented the tumor incidence, tumor volume and tumor burden in DMBA painted hamsters (groups III). No tumors were observed in control animals (Group I) and ZoARet alone administered animals (Groups IV). We have observed severe Keratosis, hyperplasia, dysplasia and squamous cell carcinoma in the buccal mucosal tissues of hamsters painted with DMBA alone (group II). A mild to moderate preneoplastic lesions (hyperplasia, keratosis and dysplasia) were noticed in group III animals.

Table 2 shows the status of plasma, erythrocytes, erythrocyte membrane and buccal mucosa TBARS in control and experimental animals in each group. The levels of TBARS were increased in plasma, erythrocytes and erythrocyte membrane and decreased in buccal mucosa of DMBA painted hamsters (Group II) as compared to control animals. Oral administration of aqueous root extract of *Zingiber officinale* at a dose of 500mg/kg-1 body weight significantly decreased the levels of TBARS in plasma, erythrocyte, erythrocyte membrane and significantly increased in buccal mucosa of DMBA painted hamsters (Group III). Hamsters treated with aqueous root extracts of *Zingiber officinale* alone showed no significant difference in TBARS as compared to control animals.

Tables 3 and 4 show the levels of circulatory, (plasma and erythrocytes) and buccal mucosa enzymatic and non-enzymatic antioxidants respectively in control and experimental animals in each group. The levels of non enzymatic antioxidants and activities of enzymatic antioxidants were significantly decreased in plasma and erythrocytes whereas disturbances in antioxidants status (Vitamin E, GSH and GPx were increased; SOD and CAT were decreased) were noticed in buccal mucosa of cancer animals as compared to control animals. Oral administration of *Zingiber officinale* Roscoe root extracts of *Zingiber officinale* at a dose of 500mg/kg-1 b.w normalized the status of antioxidants in circulation and buccal mucosal tissues. Hamsters treated with root extracts of *Zingiber officinale* alone showed no significant difference in antioxidants status as compared to control animals.

Figure 1: Histological features observed in buccal mucosa of control and experimental animals

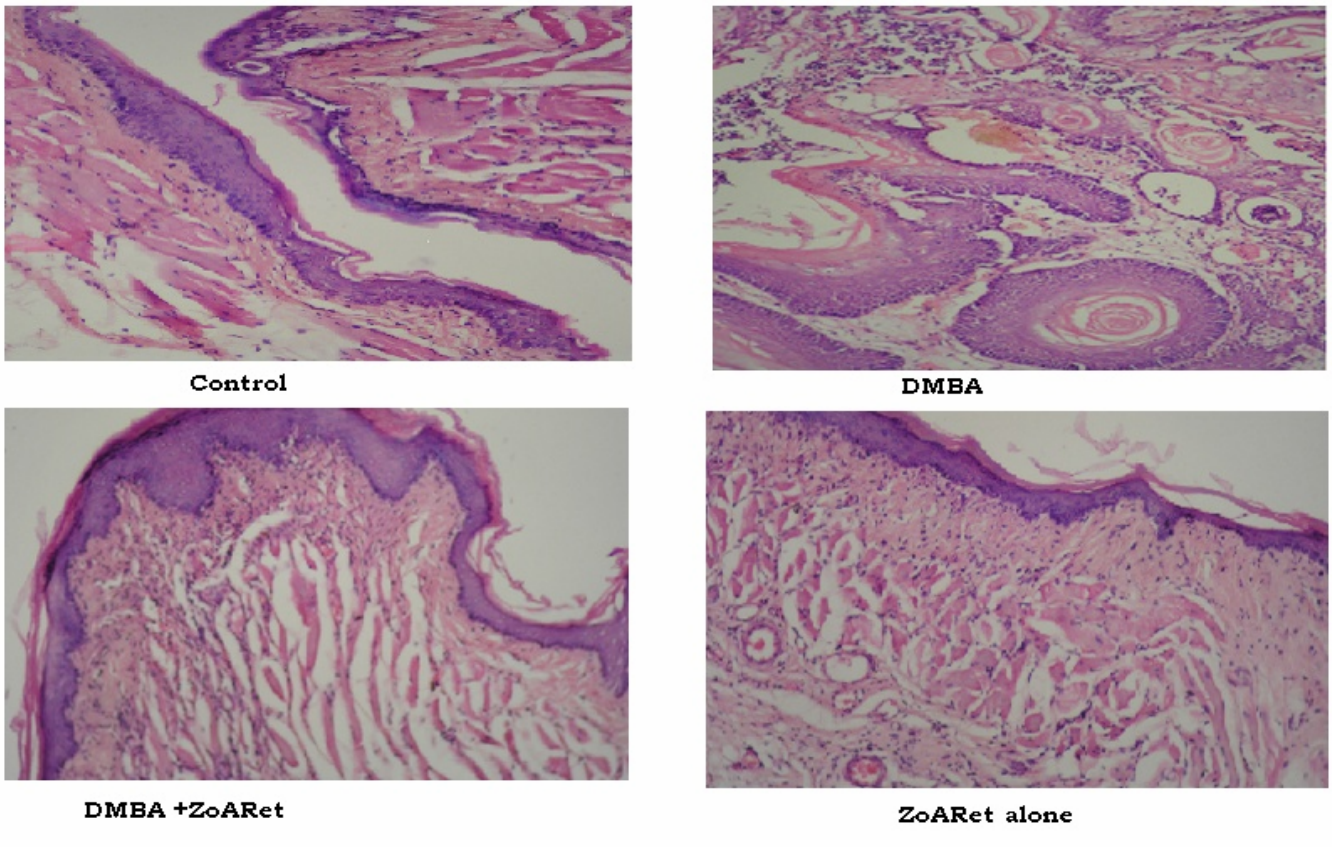

Table.1 Effect of *Zingiber officinale* root extract on squamous cell carcinoma in 0.5% DMBA painted golden Syrian hamsters.

Parameters	**Control**	**DMBA**	**DMBA+ZoERet (500mg/kg b.wt)**	**ZoERet alone (500mg/kg b.wt)**
Tumor incidence (oral Squamous cell carcinoma)	0	100 %(6)	33% 2/ (6)	0
Total number of tumors/animals	0	20(6)	4/ (2)	0
Tumor volume	0	70.37±55.9	8.07±0.49	0
Tumor burden	0	140.64±7.88	32.48±2.00	0
Keratosis	_	Severe	Mild	_
Hyperplasia	_	Severe	Mild	_
Dysplasia	_	Severe	Mild	_
Squamous cell carcinoma	–	Well differentiated squamous cell carcinoma	–	–

Values are expressed as ± SD for 6 animals in each group. Tumor volume was measuring using the $V = \frac{4}{3}\pi\left(\frac{D1}{2}\right)\left(\frac{D2}{2}\right)\left(\frac{D3}{2}\right)$ where D_1, D_2 and D_3 are the three diameters (mm) of the tumor. Tumor burden was calculated by multiplying tumor volume and the number of tumors/animal indicates ( ) total number of animals bearing tumors. ZoARet - *Zingiber officinale* Aqueous Root extract

Table 2. The levels of Thiobarbituric acid reactive substances (TBARS) in Plasma, Erythrocytes, Erythrocyte Membrane and Buccal tissue of control and experimental animals in each group.

Groups	Treatment	TBARS			
		Plasma (nmol/ml)	Erythrocytes (pmol/mg Hb)	Erythrocyte Membrane (nmol/mg protein)	Buccal tissue (nmol/mg protein)
1	Control	2.32 ± 0.30 [a]	1.90 ± 0.28 [a]	0.33 ± 0.10 [a]	69.30± 1.20 [a]
2	DMBA	4.65± 0.30 [b]	2.41 ±0.32 [b]	0.98± 0.08 [b]	41.90 ± 1.77 [b]
3	DMBA+ZoARet (500mg/kg b.wt)	3.20± 0.44 [c]	2.00 ± 0.23 [c]	0.47 ± 0.06 [c]	62.00 ±1.00 [c]
4	ZoARet alone (500mg/kg b.wt)	2.00± 0.41 [a]	1.83 ±0.10 [a]	0.32± 0.09 [a]	70.10 ± 1.50 [a]

Values are expressed as mean ± SD; n = 6. Values not sharing a common superscript significantly differ at $P < 0.05$. (DMRT)

ZoARet - *Zingiber officinale* Aqueous Root extract

Table 3. The levels of enzymatic antioxidants in plasma, erythrocytes and buccal tissue of control and experimental animals in each group

Groups	Treatment	Plasma			Erythrocyte membrane Vitamin E (μg/mg protein)	Erythrocyte GSH (mg/dl)	Buccal tissue Vitamin E (mg/100mg protein)
		Vitamin E (mg/dl)	Vitamin C (mg/dl)	GSH (mg/dl)			
1	Control	1.25 ± 0.10 [a]	1.32± 0.29 [a]	28.77± 2.63 [a]	2.30± 0.43 [a]	37.77± 1.83 [a]	1.74± 0.30 [a]
2	DMBA	0.74 ± 0.06 [b]	0.78 ± 0.13 [b]	25.10± 2.42 [b]	1.47± 0.18 [b]	21.75±2.41 [b]	2.30 ±0.20 [b]
3	DMBA+ZoARet (500mg/kg b.wt)	0.91± 0.20 [c]	1.11 ± 0.28 [c]	28.10± 2.62 [c]	1.88± 0.32 [c]	31.50± 2.84 [c]	1.96± 0.11 [c]
4	ZoARet alone (500mg/kg b.wt)	1.26 ± 0.11 [a]	1.37± 0.27 [a]	29.10± 2.29 [a]	2.36± 0.11 [a]	37.32 ±2.17 [a]	1.75 ± 0.28 [a]

Values are expressed as mean ± SD; n = 6. Values not sharing a common superscript significantly differ at $P < 0.05$. (DMRT). A - Amount of enzyme required to inhibit 50% Nitroblue tetrazolium reduction/min; B - μ moles of H_2O_2 utilized /min; C - μ moles of GSH utilized / min; D - μ moles of H_2O_2 utilized /sec. ZoARet. – *Zingiber officinale Aqueous* Root extracts

Table 4. The levels of enzymatic antioxidants in plasma, erythrocytes and buccal tissue of control and experimental animals in each group

Groups	Treatment	Plasma			Erythrocyte lysate			Buccal tissue		
		SOD (U^A/ml)	CAT (U^B/ml)	GPx (U^C/l)	SOD (U^A/mg protein)	CAT (U^D/mg Hb)	GPx (U^C/g Hb)	SOD (U^A/mg protein)	CAT (U^B/mg protein)	GPx (U^C/mg protein)
1	Control	2.45± 0.37 [a]	0.48 ± 0.03 [a]	119.70±8.50 [a]	2.16 ± 0.17 [a]	1.29 ± 0.11 [a]	14.63±0.86 [a]	4.55± 0.26 [a]	32.15± 2.78 [a]	6.60± 0.35 [a]
2	DMBA	1.43± 0.50 [b]	0.25 ± 0.02 [b]	72.05± 9.00 [b]	1.47± 0.33 [b]	0.81± 0.05 [b]	9.36± 1.22 [b]	2.75± 0.54 [b]	20.48 ±0.77 [b]	9.39± 0.95 [b]
3	DMBA+ZoARet (500mg/kg b.wt)	2.43± 0.32 [c]	0.43± 0.04 [c]	92.90± 9.67 [c]	1.97± 0.52 [c]	1.13± 0.10 [c]	12.93±1.50 [c]	3.82± 0.40 [c]	29.21± 2.77 [c]	7.32± 0.68 [c]
4	ZoARet alone (500mg/kg b.wt)	2.40± 0.33 [a]	0.51± 0.06 [a]	121.63± 10.03 [a]	2.14± 0.17 [a]	1.27±0.13 [a]	14.50±0.83 [a]	4.56± 0.22 [a]	32.45± 2.76 [a]	5.93± 0.45 [a]

Values are expressed as mean ± SD; n = 6. Values not sharing a common superscript significantly differ at $P < 0.05$. (DMRT). A - Amount of enzyme required to inhibit 50% Nitroblue tetrazolium reduction/min; B - μ moles of H_2O_2 utilized /min; C - μ moles of GSH utilized / min; D - μ moles of H_2O_2 utilized /sec

ZoARet – *Zingiber officinale* Aqueous Root extract.

Discussion

Cancer chemoprevention is currently regarded as one of the most promising avenues for cancer control (24). Recent advances in our understanding at the cellular and molecular levels of carcinogenesis have led to the development of a new promising strategy for cancer prevention that is chemoprevention (25). Chemoprevention offers a novel approach to control the incidence of oral cancer, an important contributor of cancer morbidity and mortality in the Indian subcontinent. A wide variety of phenolic substances found in our diet have been shown to exert substantial chemopreventive effects against experimental carcinogenesis (26).

DMBA, a potent carcinogen used in the present study has been reported to produce toxic and highly diffusible reactive oxygen species, capable of producing deleterious effects at sites far from the tumor (27). Reactive oxygen species are able to produce chemical modifications and to damage proteins, lipids, carbohydrates and nucleotides in the tissues (28). Reactive free radicals may damage cells by initiation of lipid peroxidation that causes profound alteration in the structural integrity and functions of cell membranes. Free radical induced lipid peroxidation has been implicated in the pathogenesis of several pathological disorders including cancer (29).

Naturally, there is a dynamic balance between the amount of free radicals generated in the body and antioxidant defense system that quench or scavenge them and thereby protect the body against pathogenesis (30). Lipid peroxides play an important role in the control of cell division. Low concentrations of oxygen free radicals have been reported to stimulate cell proliferation; whereas high levels induce cytotoxicity and cell death (31). An inverse relationship has been observed between lipid peroxidation and the rate of cell proliferation, with highly proliferating tumors showing low levels of lipid peroxidation (32). The decline of lipid peroxidation in DMBA-induced oral tumors was associated with enhanced levels of GSH, GPx and GST. GSH plays an important role in scavenging reactive oxygen species protecting cell against cytotoxic and carcinogenic chemicals (33). Enhanced lipid peroxidation associated with antioxidant depletion in circulation is a characteristic finding in malignant transformation (34).

Lowered activities of SOD and CAT enzymes were reported in patients with malignant and as well as carcinogen induced experimental carcinogenesis (34). The deficiency of ascorbic acid, vitamin E and glutathione in the circulation of tumor bearing hamsters may be due to their increased utilization to scavenge the products of lipid peroxidation. A decrease in the activities of GPx, SOD and catalase, the major cellular detoxifying enzyme systems, has been reported in malignancies (35). Enzymatic and non enzymatic antioxidants from the first and second line of defense mechanism respectively against the deleterious effects of oxidative stress induced cell damage (36).

Oral administration of *Zingiber officinale* aqueous root extracts significantly prevented the formation of oral squamous cell carcinoma in the hamster buccal pouches, which indicates its potent chemopreventive role in DMBA induced oral carcinogenesis. Although the possible mechanism include induction of phase II detoxification enzymes and increase enzymatic degradation of DMBA by liver and or enhance antioxidant defense mechanism to degrade the toxic effects of reactive oxygen species, generated by DMBA. Ginger extract was found to have immense antioxidant potential to scavenge free radicals, possibly due to its bioactive constituents, with the ability to donate electron(s) and scavenging the free radicals, specifically superoxide anions. This characteristic of plant extract was found to have a positive correlation with its antilipid peroxidation potential. Thus, the present study demonstrates the antilipidperioxidative potential of *Zingiber officinale* aqueous root extracts in DMBA induced hamster buccal pouch carcinogenesis. Further studies are needed to isolate and characterize the bioactive antioxidants principles from the root of *Zingiber officinale*.

References

1. Moore SR, Johnson NW, Pierce AM, Wilson DF. The epidemiology of mouth cancer: a review of global incidence. Oral Dis 2000; 6:65-74.
2. Blot WJ, McLaughlin JK, Devesa SS, Fraumeni JF. Cancers of the oral cavity and pharynx. In: Cancer epidemiology and prevention, Oxford University press, 1996:666-680.
3. Shklar G. Experimental oral pathology in the Syrian hamster. Progress in experimental tumor research 1972; 16: 18-38.
4. Shklar G. Development of experimental oral carcinogenesis and its impact on current oral cancer research. J Dent Res 1999; 78:1768-1772.
5. Ray G, Hussain SA. Oxidants, antioxidants and carcinogenesis. Indian J Exp Biol 2002; 40: 1213-1232.
6. Cotgreuve P, Moldens S, Orrenius D. Host biochemical defense mechanisms against prooxidants. Annu Rev Pharmacol Toxicol 1988; 28:189-212.
7. Wagner H, Hikino H. Economic and medicinal plant research. Academic Pres 1985; 230-236.
8. Dedov VN, Tron VH, Duke CC, Connor M, Christie MJ, Mondadi S, Roufogalis BD. Gingerols: a novel class of vanilloid receptor (VRI) agonists. Br J Pharmacol 2002; 137: 793-798.
9. Penna SC, Medeiros MV, Aimbire FSC. Anti-inflammatory effect of hydralcoholic extracts of *Zingiber officinale* rhizomes on rat paws and skin edema. Phytomedicine 2003; 10: 381-385.
10. Korikanthiman VS, Hedge R, Kandiannan K. Production of *Curcuma longa* L and *Zingiber officinale* in India: Indian Institute of spice Research, Cardamom Research center, 2002: 7-15.
11. Masuda Y, Kikuzaki H, Hisamoto M, Nakatani N. Antioxidant properties of gingerol related compounds from ginger. Biofactors 2004; 21:293-296.

12. Kim EC, Min JK, Kim TY, Lee SJ, Yang HO, Han S. [6]-Gingerol, a pungent ingredient of ginger, inhibits angiogenesis in vitro and in vivo. Biochem Biophys Res Commun 2005; 335: 300–308.
13. Dodge JF, Mitchell G, Hanahan DJ. The preparation and chemical characterization of hemoglobin-free ghosts of human red blood cells. Arch Biochem Biophys 1963; 100: 119-130.
14. Quist EH. Regulation of erythrocyte membrane shape by calcium ion. Biochem Biophys Res Commun 1980; 92: 631-637.
15. Yagi K. Lipid peroxides and human diseases. Chem Phys Lipids. 1987; 45: 337-351.
16. Donnan SK. The thiobarbituric acids test applied to tissues from rats treated in various ways. J Biol Chem 1950; 182: 415-419.
17. Ohkawa H, Ohisi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-358.
18. Beutler E, Kelley BM. The effect of sodium nitrate on RBC glutathione. Experientia 1963; 19: 96-107.
19. Omaye ST, Turnbull TD, Sauberlich HE. Selected method for the determination of vitamin E in animal cells. Methods Enzymol 1979; 62: 3-11.
20. Desai FD. Vitamin E analysis, methods for animal tissues. Methods Enzymol 1984; 105: 138-145.
21. Kakkar P, Das B, Visvanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biophys 1984; 21: 130-142.
22. Sinha KA. Colorimetric assay of catalase. Anal Biochem 1972; 17: 389-394.
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: 588-590.
24. Morse MA, Stoner GD. Cancer chemoprevention: principles and prospects. Carcinogenesis 1993; 14: 173-179.
25. Ferguson LR. Antimutagens as cancer chemopreventive agents in the diet. Mutat Res 1994; 307: 395-410.
26. Martins EA, Chubatsu LS, Meneghini R, Lin X, Ramamurthi K, Mishima M. Role of antioxidants in protecting cellular DNA from damage by oxidative stress. Mutat Res 1991; 250:95-98.
27. Frankel K, Wei L, Wei H. 7, 12-Dimethylbenz(a)anthracene induces oxidative DNA modifications in vivo. Free Radical Boil Med 1995; 19: 373-380.
28. De Zwart LL, Meerman JHN Commandeer S. Biomarkers of free radical damage applications in experimental animals and humans, Free Radic Biol Med 1999; 26: 202-226.
29. Draper HH, Haley M, Malondialdehyde determination as index of lipid peroxidation as index of lipid peroxidation. Methods Enzymol 1990; 186: 421-431.
30. Kolanjiappan K, Manoharan S, Kayalvizhi M. Measurement of erythrocyte lipids, lipid peroxidation, antioxidants and osmotic fragility in cervical cancer patients. Clin Chim Acta 2002; 326: 143-149.
31. Dreher D, Junod A. Role of oxygen free radicals in cancer development. Eur J of cancer 1996; 32: 30- 38.

32. Diplock AT, Rice- Evans AC, Burton RH. Is there a significant role for lipid peroxidation in the cancer prevention. Cancer Res 1994; 54: 1952-1956.
33. Saydam N, Kirb A, Demir O, Hazan E, Oto O, Saydam O. Determination of glutathione, glutathione reductase, glutathione peroxidase and glutathione S-transferase levels in human lung cancer tissues. Cancer Lett 1977; 119: 13-19.
34. Guttridge JMC. Lipidperoxidation and antioxidants as bioimarker of tissue demage. Clin Chem 1995;41:1819-1828.
35. Nagini S, Shreeram S, Ramchandran CR, Ramachandran V. Plasma concentration of lipid peroxides, β-carotene, urate and cerulo plasmin patients with oral squamous cell carcinoma. J Biochem Mol Biol Biophys 1988; 1: 235-239.
36. Manoharan S, Kolanjiappan K, Suresh K, Panjamurty K. Lipid peroxidation and antioxidant status in patients with oral squamous cell carcinoma. Ind J Med Res 2005; 122: 529-534.
37. Buzby GP, Mullen JL, Steih TP, Roasto EF. Host tumor interactions and nutrient supply. Cancer 1980; 45: 2940-2947.