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Therapeutic efficacy of green tea polyphenols on cellular thiols in 4-Nitroquinoline 1-oxide-induced oral carcinogenesis

Periasamy Srinivasan, Kuruvimalai Ekambaram Sabitha, Chennam Srinivasulu Shyamaladevi*

Department of Biochemistry, University of Madras, Guindy Campus, Chennai-600025,Tamilnadu, India

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Abstract

In cancer, a high flux of oxidants not only depletes the cellular thiols, but damages the whole cell as well. Epidemiological studies suggest green tea may mitigate cancers in human and animal models for which several mechanisms have been proposed. In the present investigation, the levels of cellular thiols such as reduced glutathione (GSH), oxidised glutathione (GSSG), protein thiols (PSH), total thiols, lipid peroxidation product conjugated dienes and the activity of gamma glutamyl transferase (GGT) were assessed in tongue and oral cavity. In 4-Nitroquinoline 1-oxide- (4-NQO) induced rats, there was a decrease in the levels of GSH, PSH and total thiols and an increase in the levels of GSSG, conjugated dienes and the activity of GGT. On supplementation of green tea polyphenols (GTP) for 30 days (200 mg/kg) for the oral cancer-induced rats, there was a moderate increase in the levels of GSH, PSH and total thiols and a decrease in the levels of GSSG, conjugated dienes and the activity of GGT. Thus, GTP reduces the oxidant production thereby maintains the endogenous low molecular weight cellular thiols in oral cancer-induced rats. From the results, it can be concluded that GTP supplementation enhances the cellular thiol status thereby mitigate oral cancer.

Keywords: Cellular thiols; Oral cancer; Green tea polyphenols; 4-Nitroquinoline 1-oxide; Lipid peroxidation

1. Introduction

Thiol compounds are known for general anti-oxidant properties such as radical quenching [1] as well as specific anti-oxidant functions. However, the most significant of the multiple roles of thiol compounds in vivo might be their critical function as cellular redox buffers, regulating protein thiol/disulfide composition. Glutathione (GSH), the major non-protein thiol in mammalian cells, is involved in many cellular functions including amino acid transport [2] and thiol-disulfide balance [3]. GSH has also been suggested as a potential regulator of protein synthesis [4]. Free sulfhydryl

* Corresponding author. Present address: New No: 66, Old No: 62, II Main Road, Gandhi Nagar, Adyar, Chennai-600025, India. Tel.: +91 44 24412575; fax: +91 44 2352494.
E-mail address: cssdevi@yahoo.com (C.S. Shyamaladevi).

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groups in protein thiols (PSH) play the role of highly reactive functional groups in biological systems and participate in several different reactions such as alkylation, arylation, oxidation, thiol-disulfide exchange etc. Therefore, the modification of PSH groups can result in severe functional damage, including loss of enzyme activity [5].

Oral squamous cell carcinoma (OSCC), the fifth most common cancer worldwide, is a major cause of morbidity and mortality [6]. The incidence rate of oral cancer in India is among the highest in the world constituting 47% of all cancers. In India, OSCC is recognized to result from chewing of betel quid containing lime, areca nut and tobacco together with smoking. In recent decades, oral cancer incidence and mortality rates have been increasing in USA, Japan, Germany and Scotland, especially among young males. In the United States, there are ∼43,000 new cases annually, resulting in ∼11,600 deaths [7].

4-NQO is known to induce multistep carcinogenesis [8]. 4-NQO is also known to induce H-*ras* mutation in chromosome 7 leading to head and neck squamous cell carcinoma in experimental murine model, which is quite similar to that of tumours that develop in tobacco chewers [9]. Carcinoma is preceded by a sequence of hyperplasia–papilloma/dysplasia–carcinoma, similar to that of human oral cancer [10]. Hence in the present study, 4-NQO is chosen as a model to investigate oral carcinogenesis.

Tea is one of the most frequently consumed beverages in the world, next to water. Catechins and epicatechins are the structural parent compounds of green tea polyphenols (GTP). The major GTP are epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), epicatechin (EC) and catechin. Over the past decade, numerous studies reported that green tea polyphenols have significant anti-carcinogenic and anti-oxidative activities, which suggest that GTP might be a useful cancer chemopreventive agent in the human populations. The biological activities of green tea are believed to be mediated by its major polyphenolic constituent, epigallocatechin gallate (EGCG). EGCG has been studied extensively due to its diverse physiological and pharmacological properties, including hypolipidemic, anti-inflammatory, anti-microbial, anti-oxidative, anti-carcinogenic and anti-tumour activities [11]. Epidemiological studies have associated the consumption of green tea with a lower risk of several types of cancer including stomach, oral cavity, esophagus and lung. In fact, tea is one of the few agents that can inhibit carcinogenesis at the initiation, promotion and progression stages [12]. The most noteworthy properties of GTP that may affect carcinogenesis are their anti-oxidative activity, inhibitory action against nitrosation reactions, modulation of carcinogenic enzymes, trapping of ultimate carcinogens, and inhibition of cell proliferation-related activities [13]. The worldwide interest in green tea as a cancer preventive agent for humans has increased since it is non-toxic and it is effective in a wide range of organs [14].

Based on the above facts, it was proposed to study the levels of cellular thiols and the activity of GGT to determine the therapeutic efficacy of GTP on 4-NQO-induced oral cancer.

2. Materials and methods

2.1. Materials

4-NQO was purchased from Sigma Chemical Company (St. Louis, MO, USA). Fresh green tea leaves were collected from The Nilgris. All other chemicals used were of analytical grade.

2.2. Preparation of GTP

Extraction of green tea polyphenols (GTP 80%) was done by adapting the procedure of Shaowen Lee (Director, Human King long Bioresource Co. Ltd., China). Green tea leaves were extracted with six times volume of 30% grain alcohol under 60–70 °C for 20 min. Filtered and cooled and the solution was extracted. The residual green tea leaves are extracted with four times volume of 30% grain alcohol. The extract was filtered and cooled, and extracts were pooled, concentrated and the grain alcohol was recovered under low temperature (<50 °C) to obtain the concentrated solution (solid >25%) and subsequently purified using ethyl acetate to get 80% GTP. From the concentrated extract, ethyl acetate was recovered and GTP was spray dried into powder. The amounts of polyphenols in the extract were estimated according to the modified method of Price and Butler [15], which accounts approximately 80% polyphenols.

2.3. Animals

Wistar strain male albino rats (10 weeks old) weighing 180–200 g were purchased from TANUVAS (Chennai, India). The animals were housed, four per cage in a room with controlled temperature and humidity with 12 h light:12 h dark cycles. All the animals were given a standard rat feed (Hindustan Lever Ltd., Bangalore) and tap water ad libitum. This study was conducted as per the guidelines of the human/animal ethical committee of our institution.

2.4. Experimental protocol

The animals were divided into four groups of 12 animals each. Group I (12) served as control, Group II (24) served as induced, oral carcinoma was induced by painting 4-NQO 0.5% in propylene glycol using No. 4 painting brush three times/week for 12 weeks, after 22 weeks, oral cancer was induced. Group III (12) served as drug control, received 200 mg of GTP/kg b.wt. p.o., for 30 days. Group IV (12 from Group II) served as treated, received 200 mg of GTP/kg b.wt. p.o., for 30 days, from 22–26 weeks.

After the experimental period, the animals were anaesthetized using ether, sacrificed by cervical decapitation. The mouth was cut opened using a surgical knife. The tongue and whole oral cavity tissue (hard palate, cheek, and floor of the mouth) was excised out, weighed and the tissues were homogenised in Tris–HCl buffer pH 7.4 and centrifuged at 3000 rpm for 10 min. The supernatant obtained was used for the various assays. Protein was estimated by the method of Lowry et al. [16]. GSH was estimated by the method of Moron et al. [17]. Virtually all the non-protein sulphydryl content of the cell is in the form of reduced gultathione.5,5[1]-dithio(2-nitrobenzoic acid) (DTNB) is a disulfide compound that is readily reduced by sulphydryl compounds forming a highly colored anion. The optical density of this yellow substance was measured at 412 nm. GSSG was measured by the method of Griffith [18]. Total thiol content was estimated by the method of Sedlack and Lindsay [19], using Ellman's reagent (DTNB), which was reduced by thiol groups to form one mole of 2-nitro 5-mercaptobenzoic acid per mole of thiol, which has an absorption maximal at 412 nm. Protein thiols were calculated by subtracting the non-protein thiols from total thiols. Conjugated dienes were estimated by the method of Klein [20]. The extent of peroxidation was determined by the measurement of conjugated dienes, which is arrived by computing the ratio of absorbance at 233 and 215 nm. GGT was assayed according to the modified method of Indrani and Hill [21]. Transpeptidation activity of GGT transfers the gamma glutamyl moiety of gamma glutamyl para nitroanilide to glycylglycine and releases the para nitroanilide, which has an absorbance maximal at 405 nm.

2.5. Statistical analysis

All data were analysed with SPSS/10 student software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by LSD. The values are expressed as mean ± S.D., P value of less than 0.001, 0.01 and 0.05 was considered to indicate statistical significance.

3. Results

Table 1 depicts the effect of GTP on 4-NQO-induced oral cancer. There was significant decrease ($P < 0.001$)

Table 1
Effect of GTP on 4-NQO-induced oral cancer

Group (treatment)	Number of animals examined	Visible tumours			
		Tumour incidence	Number of animal/animal	Tumour volume (mm^3)/animal	Tumour regression
Control	12	0	0	0	0
4-NQO	12	(9/12) 100%	4.92 ± 0.44	88.30 ± 3.05	(9/12) 100%
GTP	12	0	0	0	0
4-NQO → GTP (post)	12	(8/12) 100%	3.86 ± 0.66*	77.38 ± 4.82*	(5/8)* 62.50%

Results are expressed as mean ± S.D. Tumour volume (mm^3) was calculated by the formula-$4/3\pi r^3$(r represents the average radius of three diameter measurement in mm).

* Significant difference ($P < 0.001$, LSD) compared with group II (4-NQO).

Table 2
Effect of GTP on the levels of cellular thiols and activity of GGT in the tongue of control and experimental groups

Parameters	Group I (control)	Group II (oral cancer-induced)	Group III (drug control)	Group IV (treated)
GSH	1.15 ± 0.08	0.57 ± 0.04^{c}	1.51 ± 0.06^{NS}	0.80 ± 0.12^{a}
GSSG	0.56 ± 0.04	1.07 ± 0.05^{c}	0.59 ± 0.05^{NS}	0.91 ± 0.02^{b}
PSH	6.32 ± 0.10	3.09 ± 0.07^{c}	6.35 ± 0.08^{NS}	4.09 ± 0.07^{c}
Total thiols	7.47 ± 0.15	3.67 ± 0.07^{c}	7.50 ± 0.06^{NS}	4.89 ± 0.05^{c}
Conjugated dienes	1.62 ± 0.04	2.53 ± 0.04^{c}	1.62 ± 0.03^{NS}	1.92 ± 0.03^{c}
GGT	12.79 ± 0.57	19.41 ± 0.92^{c}	12.59 ± 0.49^{NS}	15.91 ± 0.51^{c}

Values are expressed as mean ± S.D. for six rats in each group. GSH, μg/mg protein; GSSG, μg/mg protein; PSH, μg/mg protein; total thiols, μg/mg protein; conjugated dienes, ratio of absorbance at 233 and 215 nm; GGT, IU/L. On comparing Groups II, III with Group I $^{c}P < 0.001$; NS, non-significant. On comparing Group II with Group IV $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$.

in the percentage of tumours, number of tumours and volume of tumours in GTP treated animals (Group IV) when compared to that of oral cancer-induced animals (Group II).

Table 2 shows the level of cellular thiols and the activity of GGT in the tongue of control and experimental rats. The levels of GSH, PSH and total thiols were markedly decreased ($P < 0.001$) whereas the levels of GSSG, conjugated dienes and the activity of GGT were found to be significantly increased ($P < 0.001$) in oral cancer-induced rats (Group II), when compared with control rats (Group I). In oral cancer-induced rats, supplementation of GTP for 30 days increased the levels of GSH ($P < 0.05$), PSH ($P < 0.01$) and total thiols and decreased the level of GSSG, conjugated dienes and the activity of GGT (Group IV). No significant changes were found in GTP alone treated rats (Group III) as compared with control rats.

The levels of cellular thiols and the activity of GGT in the oral cavity of control and experimental group of animals are presented in Table 3. The levels of GSSG, conjugated dienes and the activity of GGT were found to be significantly high ($P < 0.001$) and the levels of GSH, PSH and total thiols were found to be significantly lowered ($P < 0.001$) in oral cancer-induced rats when compared to control rats. Administration of GTP significantly increased the levels of GSH, PSH and total thiols and significantly reduced the levels of GSSG, conjugated dienes and the activity of GGT when compared with oral cancer-induced rats. No significant changes were observed for GTP alone treated rats, when compared with control rats.

4. Discussion

In the present investigation, decrease in the levels of GSH, PSH and total thiols and an increase in the levels of GSSG, conjugated dienes and the activity of GGT were observed in tongue and oral cavity of oral cancer-induced rats when compared with control rats. Supplementation of GTP scavenges free radicals; this might be attributed to the enhancement of cellular thiol levels.

The cell is protected against damage by several mechanisms such as oxygen consumption, primary

Table 3
Effect of GTP on the levels of cellular thiols and activity of GGT in the oral cavity of control and experimental groups

Parameters	Group I (control)	Group II (oral cancer-induced)	Group III (drug control)	Group IV (treated)
GSH	1.73 ± 0.01	0.84 ± 0.02^{a}	1.75 ± 0.04^{NS}	1.08 ± 0.06^{a}
GSSG	0.76 ± 0.03	1.52 ± 0.02^{a}	0.78 ± 0.03^{NS}	1.24 ± 0.04^{a}
PSH	9.15 ± 0.04	4.34 ± 0.17^{a}	9.34 ± 0.09^{NS}	6.45 ± 0.14^{a}
Total thiols	10.87 ± 0.05	5.53 ± 0.18^{a}	11.09 ± 0.14^{NS}	7.53 ± 0.20^{a}
Conjugated dienes	2.27 ± 0.02	3.55 ± 0.05^{a}	2.30 ± 0.02^{NS}	3.09 ± 0.02^{a}
GGT	19.99 ± 0.16	30.30 ± 0.26^{a}	19.89 ± 0.11^{NS}	23.74 ± 0.46^{a}

Values are expressed as mean ± S.D. for six rats in each group. GSH, μg/mg protein; GSSG, μg/mg protein; PSH, μg/mg protein; total thiols, μg/mg protein; conjugated dienes, ratio of absorbance at 233 and 215 nm; GGT, IU/L. On comparing Groups II, III with Group I, Group II with Group IV, $^{a}P < 0.001$; NS, non-significant.

radical scavenging, release of bound endogenous reactors, inhibition of oxygen transport etc. [22]. Among the protectors, cellular thiols are important mainly GSH plays a major role thus, GSH belongs to the second line of anti-oxidant defence which is the most abundant non-protein thiol synthesised in vivo and serves as a scavenger of different free radicals [23]. GSH has also been reported to have anti-cancer effects against experimentally induced oral cancer and as a chemopreventive agent [24]. Lipid peroxidation was also known to deplete the protein thiols. Casini et al. [25] have shown that a significant decrease in protein thiols was observed only when lipid peroxidation is developed. Oxygen free radicals and hydrogen peroxide are able to react directly with protein sulphydryl groups [26] and in the absence of glutathione this effect may become pronounced. Oxidative stress which can enhance *S*-thiolation causes formation of one nmole protein SSG/1 nmol GSSG during *t*-butylhydroperoxide oxidation of mitochondria [27]. It was also found that during the conditions of oxidative stress the PSH were depleted. This was correlated with damage to the cells [28].

In the present investigation there was a significant depletion in the GSH, PSH and total thiols. Thus, there was an increase in the levels of GSSG. This could be attributed due to the increased oxidative stress in 4-NQO-induced oral cancer. In cancer, there is an enormous production of free radicals in the system [29]. Dormandy [30] has proposed a close relationship between free radical activity and malignancy. Szatvowski and Nathan [31] reported an increase in lipid peroxidation and poor anti-oxidant system. In 4-NQO-induced oral cancer there was depletion in anti-oxidants and increase in lipid peroxidation [32] and protein peroxidation, which leads to decrease in PSH and total thiols.

In the present study, the levels of conjugated dienes were found to be increased. This might be due to the enormous production of free radicals in cancer. Lipid peroxidation is a free radical mediated process, it is involved in the formation of lipid radicals, rearrangement of unsaturated lipids that results in the variety of degraded products like alkanes, MDA, conjugated dienes and lipidhydroperoxides and eventually destruction of membrane lipids. Increase in the accumulation of conjugated dienes in the cells can result in their cellular degradation, biochemical, functional changes and even cell death [33]. Elevated levels of conjugated dienes in the oral cancer-induced rats might be due to an imbalance between pro and anti-oxidant in favour of the former, which is deleterious for the cells. On administration of GTP to the oral cancer-induced animals, there was a decrease in the lipid peroxidation because GTP is a potent anti-oxidant, which scavenges free radicals, the mediators of lipid peroxidation [34]. Thus the levels of GSH, PSH and total thiols were found to moderately maintained and there was a significant decrease in the levels of conjugated dienes (Tables 2 and 3) in both tongue and oral cavity. This could be attributed to the inhibition of tumour promotion related enzymes such as ornithine decarboxylase, protein kinase C, lipoxygenase and cyclooxygenase [35–37] by GTP.

In the present study, there was an increase in GGT activity in oral cancer-induced group II animals. GGT is a marker of neoplastic progression and occur in a number of human neoplasm's and their metastasis [38]. The physiological function of GGT is closely connected with the metabolism and transport of glutathione. GSH is exported to the blood plasma and it is broken down by GGT. The broken products are transported and utilised for GSH synthesis, inhibition of GGT also interrupts salvage pathway of GSH synthesis [39]. Supplementation of GTP significantly reduced the activity of GGT (Tables 2 and 3) in both tongue and oral cavity. Qing *et al.* [40] reported that administration of GTP in liver carcinogenesis decreased GGT positive foci by 60%. The results of the present study is consistent with the previous findings [41].

The proposed mechanism of the study is "GTP is present in the system as glucuronide conjugates which in turn directly scavenges free radicals thereby maintains the anti-oxidant status, thus preserving the thiol groups of enzymes and proteins present in the system".

In conclusion GTP a known anti-oxidant inhibits lipid peroxidation, which in turn moderately increased the level of cellular thiol status and decreased the activity of GGT, a marker of tumour progression.

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References

[1] S.M. Deneke, Thiols based anti-oxidant, Curr. Top. Cell. Regul. 36 (2000) 151–181.
[2] A. Meister, On the enzymology of amino acid transport, Science 180 (1973) 33–39.
[3] D.M. Ziegler, Role of reversible oxidation-reduction of enzyme thiols disulfides in metabolic regulation, Ann. Rev. Biochem. 54 (1985) 305–329.
[4] N.S. Kosower, E.M. Kosower, The glutathione status of cells, Int. Rev. Cytol. 54 (1978) 109–160.
[5] S. Orrenius, Biochemical mechanisms of cytotoxicity, Trends Pharmacol. Sci. 6 (1985) 15–20.
[6] S.R. Moore, N.W. Johnson, A.M. Pierce, D.F. Wilson, The epidemiology of mouth cancer: a review of global incidence, Oral Dis. 6 (2000) 65–74.
[7] C. Boring, T.S. Squrils, T. Tong, Cancer statistic, CA, Cancer J. Clin. 2 (1992) 19–38.
[8] T. Sugimura, Multistep carcinogenesis: a 1992 prospective, Science 258 (1992) 603–607.
[9] F.J. Hendler, B. Yuan, M.N. Oechlsi, E.J. Lentesh, J.C. Menes, A.J. Shum-Siu, L.H. Hu, B. Hawkins, B.W. Heniford, 4-NQO oral carcinogenesis, a murine model of human head and neck cancer in: J.A. Waerner, B.M. Lippert, H.H. Rudert (Eds.), Head and Neck Cancer—Advances in Basic Research, Elsevier science, New york, 1996, pp. 79–87.
[10] G. Stenman, Malignancy of 4-NQO induced oral squamous cell carcinomas in rat, Acta Otolaryngol. 92 (1981) 557–561.
[11] H.L. Lung, W.K. Ip, C.K. Wong, N.K. Mak, Z.Y. Chen, K.N. Leung, Anti-proliferative and differentiation-inducing activities of the green tea catechin epigallocatechin-3-gallate (EGCG) on the human eosinophilic leukemia EoL-1 cell line, Life Sci. 72 (2002) 257–268.
[12] S.K. Katiyar, H. Mukhtar, Tea in chemoprevention of cancer; epidemiological and experimental studies, Int. J. Oncol. 8 (1996) 221–238.
[13] C.S. Yang, S. Kim, G. Yang, J. Lee, J.Y. Chung, C. Ho, Inhibition of carcinogenesis by tea: bioavailability of tea polyphenols and mechanisms of actions, in: Proceedings of the Society for Experimental Biology and Medicine, vol. 220, 1999, pp. 213–217.
[14] M. Suganuma, S. Okabe, N. Sueoka, E. Sueoka, S. Matsuyama, K. Imai, K. Nakachi, H. Fujiki, Green tea and cancer chemoprevention, Mut. Res. 428 (1999) 339–344.
[15] M.L. Price, L.G. Butler, Rapid visual estimation and spectrometric determination of tannine content of sorghum grain, J. Agric. Food Chem. 25 (1977) 1268–1273.
[16] O.H. Lowry, N.J. Rosenbrough, A.L. Farr, R.J. Randall, Protein measurement with the folin phenol reagent, J. Biol. Chem. 134 (1951) 265–275.
[17] M.S. Moron, J.W. Depierre, B. Mannervik, Levels of glutathione, glutathione reductase and glutathione-*S*-transferase activities in rat lung and liver, Biochim. Biophys. Acta 582 (1979) 67–70.
[18] O.W. Griffth, Determination of glutathione, glutathione disulfide using glutathione reductase 2-vinylpyridne, Anal. Biochem. 106 (1980) 207–212.
[19] J. Sedlack, R.H. Lindsay, Estimation of total, protein bound and non-sulphydryl groups in tissue with Ellman's reagent, Anal. Biochem. 25 (1968) 192.
[20] R.A. Kelin, The detection of oxidation in liposome preparations, Biochim. Biophys. Acta 210 (1970) 486.
[21] N. Indirani, P.G. Hill, Partial purification and some properties of gamma glutamyl transpeptidase from human bile, Biochim. Biophys. Acta 483 (1977) 57.
[22] C.J. Koch, C.C. Stobbe, P. Hettiarachi, Combined radiation protective and radiation sanitizing agents: IV. Measurement of intracellular protector concentrations, Int. J. Radiat. Oncol. Biol. Phys. 16 (1989) 1025–1027.
[23] G. Ray, S.A. Husian, Oxidant, antioxidants and carcinogenesis, Indian J. Exp. Biol. 40 (2002) 1213–1232.
[24] G. Shklar, J. Schwartz, D. Trickler, S.R. Cheverie, The effectiveness of a mixture of beta-carotene and ascorbic acid for cancer prevention, Nutr. Cancer 20 (1993) 145–151.
[25] A.F. Casini, E. Maellaro, A. Pompella, M. Ferrali, M. Comporti, Lipid peroxidation, protein thiols and calcium homeostasis in bromobenzene induced liver damage, Biochem. Pharmacol. 36 (1987) 3689.
[26] G. Bellomo, F. Mirabelli, P. Richelmi, Orrenius, Critical role of sulphydryl groups in ATP-dependent ca^{2+} sequestration by the plasma membrane fraction from rat liver, FEBS Lett. 163 (1983) 136.
[27] T.E. Creighton, Biochemical, physiological, toxicological and clinical aspects in: A. Larsson, S. Orrenins, A. Holmgren, B. Mannervik (Eds.), Function of Glutathione, Raven Press, New York, 1983, pp. 205–213.
[28] M. Irshad, P.S. Chaudhuri, Oxidant-antioxidant system: role and significance in human body, Indian J. Exp. Biol. (2002) 40.
[29] P.A. Cerutti, Oxyradicals and cancer, Lancet 344 (1994) 862–868.
[30] T.L. Dormandy, An approach to free radicals, Lancet 1 (1983) 1010–1014.
[31] T.P. Szatrowski, C.F. Nathan, Production of large amount of H_2O_2 by human tumour cells, Cancer Res. 51 (1991) 794–798.
[32] S. Balasenthil, C.R. Ramachandran, S. Nagini, Prevention of 4-Nitroquinoline-1-oxide rat tongue carcinogenesis by garlic, Fitoterapia 72 (2001) 524–531.
[33] C.D. Upasani, A. Khera, R. Balaraman, Effect of lead and vitamin E, C or spiruline on malandialdehyde, conjugated dienes and hydro peroxides in rats, Indian J. Exp. Biol. 39 (2001) 70–74.
[34] E. Siazydlewska, J. Ostrowska, R. Farbiszewski, K. Michalak, Protective effect of green tea against lipid peroxidation in the rat liver, blood, serum and brain, Phytomedicine 9 (2002) 232–238.
[35] M.T. Huang, C.T. Ho, Z.Y. Wang, T. Ferraro, T. Finnegan-Oliver, Y.R. Lou, J.M. Mitchel, Inhibitory effect of topical application of a green tea polyphenol fraction on tumour initation and promotion in mouse skin, Carcinogenesis 13 (1992) 947–954.
[36] Z.Y. Wang, R. Agarwal, D.R. Bickers, H. Mukthar, Protection against ultraviolet B radiation-induced photocarcinogenisis in hairless mice by green tea polyphenols, Carcinogenisis 12 (1991) 1527–1530.
[37] S. Yoshizawa, T. Horiuchi, M. Sugimura, Penta-*o*-gallyl-β-D-glucose and (−)-epigallacatechin gallate: cancer prevention

agent in: M.T. Huang, C.T. Ho, C.Y. Lee (Eds.), Phenolic Compounds in Foods and Health. II: Antioxidant and Cancer Prevention, American Chemical society, Washington, 1992, pp. 316–325.

[38] G.P. Sachdev, G. Wen, B. Martin, G.S. Kishore, O.F. Fox, Effects of dietary fat and α-tocopherol on gamma glutamyl transferase activity in 7, 12,-dimethyl benz(a)anthracene induced mammary gland adenocarcinomas, Cancer Biochem. Biophys. 5 (1980) 15–23.

[39] E. Ristof, A. Larrson, Patients with genetic defects in gamma glutamyl cycle, Chem. Biol. Interact. 11 (1998) 113–121.

[40] G. Qing, Y. Ning, P.D. Lotikar, Chemoprevention of aflatoxin B1 initiated and carbon tetrachloride promoted hepatocarcinogenesis in the rat, Nutr. Cancer 38 (2000) 215–222.

[41] Y. Li, R.Q. Yan, G.Z. Qin, L.L. Qin, X.X. Daun, Reliability of short term lost for hepatocarcinogenesis induced by aflatoxin B1, IARL Sci. Pub. 105 (1991) 431–433.