Pharmacological Reports
2006, 58, 229–235
ISSN 1734-1140

Prevention of 1,2-dimethylhydrazine-induced circulatory oxidative stress by bis-1,7-(2-hydroxyphenyl)-hepta-1,6-diene-3,5-dione during colon carcinogenesis

Thiyagarajan Devasena[1], Venugopal P. Menon[2], Kallikat N. Rajasekharan[3]

[1]Department of Biotechnology, Mother Teresa Women's University, Kodaikanal 624 102, Tamil Nadu, India

[2]Department of Biochemistry, Faculty of Science, Annamalai University, Annamalainagar 608 002, Tamil Nadu, India

[3]Department of Chemistry, University of Kerala, Trivandrum, India

Correspondence: Thiyagarajan Devasena, e-mail: tdevasenabio@yahoo.com

Abstract:
We have performed this study to investigate the modulatory effect of bis-1,7-(2-hydroxyphenyl)-hepta-1,6-diene-3,5-dione, a bisdemethoxy curcumin analog (BDMCA) on circulatory lipid peroxidation (LPO) and antioxidant status during 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis in male Wistar rats. The effects were compared with that of the reference drug, curcumin. Increased tumor incidence as well as enhanced LPO in the circulation of tumor bearing rats was accompanied by a significant decrease in the level of reduced glutathione and activities of glutathione peroxidase (GPx), glutathione S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT). Intragastric administration of BDMCA or curcumin to DMH-treated rats significantly decreased colon tumor incidence and the circulatory LPO, with simultaneous enhancement of GSH content and GPx, GST, SOD and CAT activities. We report that BDMCA exert its chemopreventive effect by decreasing the colon tumor incidence as well as by modulating circulatory oxidative stress in DMH-treated rats through its influence on LPO and antioxidant status. The effects of BDMCA were comparable with that of the reference compound curcumin, a well known anticarcinogen and antioxidant. Thus, it would be suggested that the methoxy group is not responsible for the beneficial effects, however, the terminal phenolic moieties or the central 7-carbon chain may play a role.

Key words:
colon cancer, curcumin analog, dimethylhydrazine, chemoprevention, lipid peroxidation, antioxidant

Introduction

Oxidative stress, an imbalance in oxidant-antioxidant status is characterized by an increase in lipid peroxidation (LPO) and a decrease in activities of antioxidant enzymes [6, 26]. Oxidative stress is favored by a high content of unsaturated lipid substrates, aerobic conditions and presence of metal ions. In red blood cells (RBCs), the content of unsaturated lipids is high, oxygen concentration is even more enhanced and iron, the potent peroxidation catalyst is abundant. Therefore, RBCs are most affected by oxidative stress [7, 23]. 1,2-Dimethylhydrazine (DMH), a potent colon carcinogen undergoes oxidative metabolism resulting in the production of electrophilic diazonium

ion, which is known to elicit oxidative stress in the RBCs [15, 17]. Circulatory LPO and antioxidant status could, therefore, be used as markers of chemoprevention by natural and synthetic compounds. Studies from this laboratory [8] and by other researchers [2, 3, 22, 32] have demonstrated the importance of circulatory lipid peroxidation and antioxidant changes in monitoring the chemopreventive and antioxidative effects of chemopreventive agents.

Recently, we demonstrated the chemopreventive [10] and antioxidative [9] effects of a synthetic compound, bis-1,7-(2-hydroxyphenyl)-hepta-1,6-diene-3,5-dione (bisdemethoxy curcumin analog – BDMCA). We have reported that the synthetic compound BDMCA is as effective as the natural compound curcumin (bis-1,7-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene-3,5-dione) in 1) reducing the colon tumor incidence and 2) ameliorating hepatic oxidative stress in DMH-treated rats. This study was undertaken to examine whether BDMCA exhibits antioxidant activity by investigating its effect on DMH-induced circulatory oxidative stress during colon carcinogenesis. Oxidative stress was assessed by measuring lipid peroxidation, detoxification enzyme and antioxidant enzyme activities.

Materials and Methods

Animals

Male Wistar rats with body weight between 100–120 g were obtained from the Central Animal House, Department of Experimental Medicine, Annamalai University, Tamil Nadu, India and kept at 30 ± 2°C under 12-h light/12-h dark cycles. Commercial pellet diet containing 5% fat (Hindustan Lever Ltd., Mumbai, India) was powdered and mixed with 15% peanut oil, making a total of 20% fat in the diet. This diet was fed to the rats throughout the experiment and water was given *ad libitum.*

Drugs

DMH (1,2-dimethylhydrazine) and curcumin were purchased from Sigma Chemical Company, USA. BDMCA was synthesized by the method of Dinesh Babu and Rajasekharan and dissolved in DMSO [11].

Experimental protocol

Group	Treatment schedule
1. Control	–
2. DMH	Weekly subcutaneous injection of DMH (dissolved in EDTA) (20 mg/kg) for 15 weeks
3. BDMCA	Daily oral administration of BDMCA (80 mg/kg) for 30 weeks
4. DMH + BDMCA	DMH as in group 2 and BDMCA as in Group 3
5. Curcumin	Daily oral administration of curcumin (80 mg/kg) for 30 weeks
6. DMH + curcumin	DMH as in Group 2 and curcumin as in Group 5

The procedures employed in the study was accepted and approved by Ethical Committee for Animal Research.

Blood sampling

Blood was collected into heparinized tubes and plasma was separated by centrifugation at 2,000 × g for 10 min.

Tab. 1a. Incidence of colonic neoplasms

Group	Number of rats examined	Number of rats with tumor	Number of tumors per rat (average)	Tumor size (average, cm)	Tumor incidence (%)
Control	6	0	0	–	0
DMH	6	6	6	≈2	100
BDMCA	6	0	0	–	0
DMH + BDMCA	6	3	1	< 0.5	16.66
Curcumin	6	0	0	–	0
DMH + curcumin	6	1	1	< 0.5	16.66

Tab. 1b. Microscopical observations of colon

Observations	Control	DMH	BDMCA	DMH + BDMCA	Curcumin	DMH + curcumin
Mucin	–	Lumen is dilated and filled with mucin in which clumps of tumor cells afloat	–	–	–	–
Submucosa	Normal	–	Normal	Dense lymphocyte infiltration organized as aggregates	Normal	Dense lymphocyte infiltration organized as aggregates
Adenocarcinoma	–	Well differentiated	–	–	–	–
Papillary pattern	–	Large number of papillae	–	–	–	–
Dysplasia	–	Marked	–	–	–	–
Cellular pleomorphism	–	Marked	–	–	–	–

Preparation of hemolysate

After the separation of plasma, the buffy coat was removed and packed cells (RBCs) were washed thrice with cold physiological saline. To determine the activity of RBC antioxidant enzymes, RBC lysate was prepared by lysing a known volume of RBCs with cold hypotonic phosphate buffer, pH 7.4. The hemolysate was separated by centrifuging at 3,000 × g for 10 min at 2°C.

Biochemical investigation

LPO as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) was measured in plasma by the method of Yagi [34] and in RBCs (red blood cells) by the method of Donnan [13]. RBC reduced glutathione (GSH) was assessed according to Ellman's method [14]. Activity of glutathione peroxidase (GPx) was assayed by the method of Rotruck et al. [27]. Glutathione S-transferase (GST) activity was measured by the method of Habig et al. [19]. Superoxide dismutase (SOD) was estimated by the method of Kakkar et al. [21] and catalase (CAT) by the method of Sinha [28].

Statistical analysis

Data from biochemical investigation were analyzed using analysis of variance (ANOVA) and the group means were compared by Duncan's Multiple Range Test (DMRT). The results were considered statistically significant if the "p" value was 0.05 or less.

Results

Incidence of colonic tumor in different groups is shown in Table 1. The incidence of colonic neoplasms in DMH-administered group was 100%. However, the incidence was reduced to 16.66% in DMH + BDMCA and DMH + curcumin groups (Group 4 and 6). In BDMCA- and curcumin-treated rats (Group 3 and 5) no tumor was noticed.

The levels of LPO in plasma and RBCs of control and experimental groups are given in Table 2. LPO was significantly the highest in DMH group, but significantly lower in DMH + BDMCA and DMH + curcumin group when compared to DMH group. However, no significant difference was noticed between BDMCA and curcumin-treated control group.

Tab. 2. Levels of thiobarbituric acid reactive substances (a measure of LPO) in plasma and red blood cells

Group	Plasma (nmol/ml)	Red blood cells (pmol/mg Hb)
Control	2.72 ± 0.08	2.06 ± 0.03
DMH	4.76 ± 0.13*	3.72 ± 0.25*
BDMCA	2.10 ± 0.06	1.45 ± 0.08
DMH + BDMCA	2.78 ± 0.05#	2.12 ± 0.10#
Curcumin	2.08 ± 0.10	1.33 ± 0.06
DMH + curcumin	2.81 ± 0.11	2.19 ± 0.15

Values are the mean ± SD from 6 rats in each group. * DMH group values differs significantly from control group ($p < 0.05$). # DMH + BDMCA group differs significantly from DMH group ($p < 0.05$)

Tab. 3. Circulatory GSH content and activities of GPx, GST, SOD and CAT

Group	GSH (mg/dl)	GPx (unit[1])	GST (unit[2])	SOD (unit[3])	CAT (unit[4])
Control	35.42 ± 3.08	24.44 ± 3.16	3.58 ± 0.30	3.52 ± 0.47	2.79 ± 0.12
DMH	24.76 ± 2.26*	15.31 ± 3.04*	1.10 ± 0.04*	2.14 ± 0.11*	1.23 ± 0.05*
BDMCA	41.25 ± 2.05	32.35 ± 3.33	4.73 ± 0.33	4.62 ± 0.31	3.91 ± 0.09
DMH + BDMCA	32.13 ± 5.42#	21.13 ± 4.07#	3.26 ± 0.31#	3.20 ± 0.30#	2.76 ± 0.09#
Curcumin	41.28 ± 2.22	33.41 ± 3.56	4.52 ± 0.32	4.67 ± 0.29	3.52 ± 0.12
DMH + curcumin	33.09 ± 5.26	21.96 ± 4.33	3.19 ± 035	3.23 ± 0.28	2.75 ± 0.10

Values are the mean ± SD from 6 rats in each group. * DMH group values differs significantly from control group ($p < 0.05$). # DMH + BDMCA group differs significantly from DMH group ($p < 0.05$). Unit[1]: µg of GSH utilized/min/mg hemoglobin; Unit[2]: µM of CDNB-GSH conjugate formed/min/mg hemoglobin; Unit[3]: Enzyme required for 50% inhibition of NBT reduction; Unit[4]: µM of H_2O_2 utilized/min/mg of hemoglobin

Table 3 summarizes the GSH content and activities of GPx, GST, SOD and CAT in RBC lysate of animals in each group. The level of GSH as well as the activities of all the four enzymes were significantly lowered in DMH-treated animals when compared with control animals, whereas the GSH content and the enzyme activities were significantly increased to near normal values in DMH + BDMCA and DMH + curcumin-treated animals. BDMCA- and curcumin-treated animals showed no significant difference in any of these parameters.

Discussion

The reduced incidence of colonic neoplasms in DMH + BDMCA group (16.66%) and DMH + curcumin group (16.66%) when compared to DMH group (100%) shows that: i) BDMCA exhibits anticarcinogenic activity, ii) BDMCA is as effective as curcumin in the anticarcinogenic activity. Moreover, 0% tumor incidence in BDMCA-treated control group suggests that BDMCA causes no disruption of normal cellular homeostasis, and hence it is non-toxic.

DMH is a procarcinogen which induces colon tumor formation after undergoing various metabolic changes in the colon and liver [15–17, 25]. Blocking agents are chemopreventive agents that prevent tumorigenesis by blocking the carcinogen-DNA adduct formation [18] (Fig. 2). It has been reported that phenolic compounds like ferulic acid, caffeic acid and curcumin act as blocking agents and prevents carcinogenesis [18]. From Figure 1 it is clear that BDMCA also possesses phenolic group and mimics our reference compound curcumin. Hence, we suggest that anticarcinogenic action of BDMCA may be due to the terminal phenolic group which could act as a blocking agent.

Anto et al. [1] have reported that the existence of *ortho* hydroxyl (*o*-OH) group in the benzene ring of BDMCA is critical for the prevention of TPA-induced skin cancer in mice. Therefore, we could suggest that the *o*-OH group of BDMCA may be responsible for the prevention of DMH-induced colon cancer thereby leading to a decrease in its incidence.

Our study investigated the 1) levels of LPO, in terms of thiobarbituric acid reactive substances (TBARS), 2) activities of detoxifying enzymes and 3) activities of antioxidant enzymes in the RBCs which are considered to be more sensitive to oxidative stress [7, 23]. Enhanced LPO associated with depletion in detoxifying (GPx and GST) and antioxidant (CAT, SOD) enzymes in the RBCs were observed in our study. These are the characteristic findings in malignant transformation [7, 26]. It is evident from these results that, in DMH-treated rats, the oxidant-antioxidant homeostasis is disturbed.

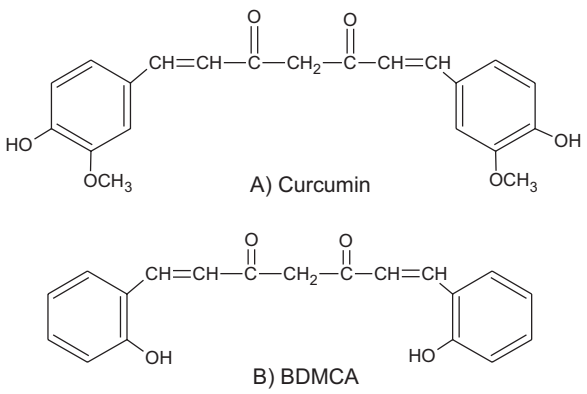

Fig. 1. Structure of curcumin and curcumin analog

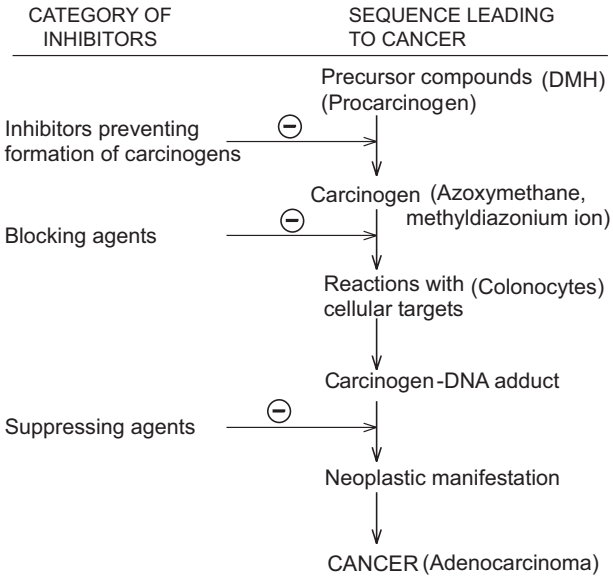

Fig. 2. Cancer inhibitors and stage of inhibition

LPO was found to be increased significantly in the plasma and RBCs of DMH-treated rats when compared to control rats. DMH, a procarcinogen undergoes oxidative metabolism in the liver which results in the production of active carcinogenic electrophile (diazonium ion) that is released into the circulation eventually leading to LPO in plasma and RBCs [5].

Further, Szatrowski and Nathan [30] have suggested that tumor cells produce substantial amount of H_2O_2 that is released into the circulation. Thus, the increased susceptibility of plasma and RBCs of DMH-treated rats could be due to the production of H_2O_2 by the tumor cells.

The decreased GSH content and a fall in the activities of GPx and GST were noticed in RBCs of DMH-treated rats. GSH is a natural thiol that exhibits anticarcinogenic and antioxidant properties [22]. Therefore, the deficiency of GSH in circulation of DMH-treated rats observed in our study may be due to the increased utilization of GSH to counteract LPO.

GPx and GST are detoxification/biotransformation enzymes which are involved in the detoxification of toxic substances such as xenobiotics, carcinogens, free radicals and peroxides by conjugating these substances with GSH [20]. Since the ultimate carcinogenic form of DMH is a toxic electrophile (methyl diazonium ion and carbonium ion), GPx and GST take considerable significance in promoting carcinogen detoxification [4]. Thus, decrease in GPx and GST activities in RBC lysate of tumor-bearing rats may be due to their utilization in detoxification of carcinogenic metabolites of DMH.

Administration of BDMCA to DMH-treated rats decreased LPO and increased the activities of GPx and GST in the circulation. Previously, Sreejayan and Rao [29] have also reported that BDMCA is a potent inducer of detoxification enzymes. Our findings also correlate with the view of Van Leishout et al. [32] that inhibitors of carcinogenesis have an enhancing effect on carcinogen detoxifying enzymes. GPx and GST enhances the biotransformation of carcinogens [4, 20]. Induction of enzymes that are involved in the biotransformation of carcinogens may accelerate the metabolic disposal of carcinogens [3, 8, 20, 30]. Hence, induction of GPx and GST by BDMCA in DMH + BDMCA-treated rats might lead to metabolic disposal of DMH and its metabolites, resulting in the protection of RBCs and simultaneous inhibition of colon tumorigenesis. Our results also obviously suggest that the detoxifying capacity of BDMCA is comparable with that of our reference compound curcumin. Curcumin has been reported to act as detoxifying agent through its β-diketone moiety located in the central 7-carbon chain (Fig. 1A). BDMCA resembles curcumin and possesses a β-diketone moiety [12] (Fig. 1B). It could, therefore, be suggested that the β-diketone moiety of BDMCA may be responsible for increasing the GPx and GST activities in the RBCs of DMH + BDMCA-treated rats.

As mentioned earlier [30], tumor cells produce substantial amount of H_2O_2 which is released into the circulation. Accumulation of H_2O_2 results in formation of hydroxyl radical ($OH^{\bullet}$) and/or a highly toxic hypochlorous acid formed as a result of metabolism of H_2O_2 by circulatory neutrophil-derived myeloperoxidase [33]. Thus, H_2O_2 produced by tumor cells elicits an oxidative stress in RBCs. SOD and CAT are primary antioxidant enzymes reported to be more sensitive to oxidative stress [24, 31]. Therefore, the decreased activities of SOD and CAT in DMH-treated rats may be due to this oxidative stress.

Restoration of SOD and CAT activities in the circulation of DMH + BDMCA-administered rats shows that BDMCA normalizes the circulatory antioxidant status and ameliorates DMH-induced oxidative stress. Our findings correlate with the hypothesis that chemopreventive agents act as anticarcinogenic compounds by modulating carcinogen-induced circulatory oxidative stress [2, 3, 8, 10].

Conclusion

BDMCA decreases the DMH-induced colon cancer incidence and normalizes the oxidative stress in the circulation by raising detoxifying and antioxidant enzymes. The effects were comparable with that of the reference compound, curcumin. Our findings suggest that the methoxy group is not responsible/essential for the anticarcinogenic/antioxidant properties, however, the phenolic group or the β-diketone moiety in the central 7-carbon chain may be responsible. Finally, it could be concluded that increasing the activities of detoxifying and antioxidant enzymes may be one of the mechanism by which BDMCA nullifies the deleterious effects of DMH.

References:

1. Anto RJ, George J, Dinesh Babu KV, Rajasekharan KN, Kuttan R: Antimutagenic and anticarcinogenic activity of natural and synthetic curcuminoids. Mutat Res, 370, 1996, 127–131.
2. Arivazhagan S, Balasenthil S, Nagini S: Modulatory effects of garlic and neem leaf extracts on circulating lipid peroxides and antioxidants during N-methyl-N'-nitro-N-nitrosoguanidine-induced gastric carcinogenesis. Med Sci Res, 1999, 27, 527–529.
3. 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, 429–433.
4. Beckett GJ, Hayes JD: Glutathione S-transferases: Biomedical application. Adv Clin Chem, 1993, 30, 281–380.
5. Bobek P, Galbavy S, Mariassova M: The effect of red beet (*Beta vulgaris* var. Rubra) fiber on alimentary hypercholesterolemia and chemically induced colon carcinogenesis. Nahrung, 2000, 44, 184–187.
6. Cerutti PA: Oxyradicals and cancer. Lancet, 1994, 344, 862–863.
7. Della Rovere F, Granata A, Saija A, Broccio M, Tomaino A, Zirilli A, De-caridi G, Broccio G: –SH groups and glutathione in cancer patients blood. Anticancer Res, 2000, 20, 1595–1598.
8. Devasena T, Rajasekaran KN, Gunasekaran G, Viswanathan P, Menon VP: Anticarcinogenic effect of bis-1,7-(2-hydroxy- phenyl)-hepta-1,6-diene-3,5-dione, a curcumin analog on DMH-induced colon cancer model. Pharmacol Res, 2003, 47, 133–140.
9. Devasena T, Menon VP: Enhancement of circulatory antioxidants by fenugreek during 1,2-dimethylhydrazine-induced rat colon carcinogenesis. J Biochem Mol Biol Biophys, 2002, 6, 289–292.
10. Devasena T, Rajasekharan KN, Menon VP: Bis-1,7-(2-hydroxyphenyl)-hepta-1,6-diene-3,5-dione (a curcumin analog) ameliorates DMH-induced hepatic oxidative stress during colon carcinogenesis. Pharmacol Res, 2002, 46, 39–45.
11. Dinesh Babu KV, Rajasekharan KN: Simplified conditions for the synthesis of curcumin I and other curcuminoids. Org Procedure Int, 1994, 26, 674–677.
12. Dinkova-Kostova AT, Talalay P: Relation of structure of curcumin analogs to their potencies as inducers of phase 2 detoxification enzymes. Carcinogenesis, 1999, 20, 911–914.
13. Donnan SK: The thiobarbituric acid test applied to tissues from rats treated in various ways. J Biol Chem, 1950, 182, 415–419.
14. Ellman GL: Tissue sulphydryl groups. Arch Biochem Biophys, 1959, 82, 70–77.
15. Fiala ES, Kulakis C, Christiansen G, Weisburger JH: Inhibition of the metabolism of the colon carcinogen, azoxymethane, by pyrazole. Cancer Res, 1978, 38, 4515–4521.
16. Fiala ES, Sohn OS, Hamilton SR: Effects of chronic dietary ethanol on the *in vivo* and *in vitro* metabolism of methylazoxymethanol and methylazoxymethanol induced DNA methylation in the rat colon and liver. Cancer Res, 1987, 47, 5939–5943.
17. Fiala ES: Investigations into the metabolism and mode of action of the colon carcinogen 1,2-dimethylhydrazine and azoxymethane. Cancer, 1977, 40, 2436–2445.
18. Greenwald P: From carcinogenesis to clinical interventions for cancer prevention. Toxicology, 2001, 166, 37–45.
19. Habig WH, Pabst MJ, Jakoby WB: Glutathione S-transferase, the first enzymatic step in mercapturic acid formation. J Biol Chem, 1974, 249, 7130–7139.
20. Hayes JD, Pulford DJ: The glutathione S-transferase supergene family: Regulation of GST and contribution of the isoenzymes to cancer chemoprevention and drug resistance. Crit Rev Biochem Mol Biol, 1995, 31, 445–600.
21. Kakkar P, Das B, Viswanathan PN: A modified spectrophotometric assay of superoxide dismutase. Ind J Biochem Biophys, 1984, 21, 130–132.
22. Lii CK, Ko YJ, Chiang MT, Sung WC, Chen HW: Effect of dietary vitamin E on antioxidant status and antioxidant enzyme activities in Sprague-Dawley rats. Nutr Cancer, 1998, 32, 95–100.
23. Marlin J, Macief J, Michael J, Szcypka J, Gajewska J, Kitta T: Antioxidant defence of red blood cells and plasma in stored human blood. Clin Chim Acta, 1997, 267, 129–142.
24. Moghadasian MH, Freeman HJ, Godin DV: Endogenous antioxidant status in neoplastic and adjuvant tissues in 1,2-dimethylhydrazine-induced colon cancer in rats: Effect of oslazine. Carcinogenesis, 1996, 17, 983–987.
25. Oraevec CT, Jones CA, Huberman E: Activation of the colon carcinogen 1,2-dimethylhydrazine in a rat colon cell-mediated mutagenesis assay. Cancer Res, 1986, 46, 5068–5071.
26. Ray G, Batra S, Shukla NK, Deo S, Raina V, Ashok S, Hussain S: Lipid peroxidation, free radical production and antioxidant status in breast cancer. Breast Cancer Res, 2000, 53, 163–170.

27. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG: Biochemical roles as a component of glutathione peroxidase. Science, 1973, 179, 588–590.
28. Sinha AK: Colorimetric assay of catalase. Anal Biochem, 1972, 47, 389–394.
29. Sreejayan R, Rao MNA: Nitric oxide scavenging by curcuminoids. J Pharm Pharmacol, 1997, 49, 105–107.
30. Szatrowski TP, Nathan CF: Production of large amounts of hydrogen peroxide by human tumor cells. Cancer Res, 1991, 51, 794–798.
31. Taniguchi M, Yasutake A, Takedomi K, Inoue K: Effects of N-nitrosodimethylamine (NDMA) on the oxidative status of rat liver. Arch Toxicol, 1999, 73, 141–146.
32. Van Leishout EMM, Peters WHM: Nonsteroidal anti-inflammatory drugs enhance glutathione S-transferases of the rat digestive tract. Clin Chem Enz Commun, 2000, 8, 449–453.
33. Weiss SJ, Lo Buglio AF: Biology of diseased phagocyte generated oxygen metabolites and cellular injury. Lab Invest, 1982, 47, 5–18.
34. Yagi K: Lipid peroxides and human disease. Chem Physiol Lipids, 1978, 45, 337–351.

Received:
October 21, 2004; in revised form: January 20, 2006.