PHYTOTHERAPY RESEARCH
Phytother. Res. **14**, 347–351 (2000)

Chemopreventive Activity of Quercetin During Carcinogenesis in Cervix Uteri in Mice

Sarmishtha De, Jamuna Chakraborty, R. N. Chakraborty and Sukta Das*

Department of Cancer Chemoprevention, Chittaranjan National Cancer Institute, Calcutta - 700 026, India

The chemopreventive action of quercetin was examined during 20-methyl cholanthrene induced cervical neoplasia in virgin Swiss albino mice. The effects were evaluated on the basis of histopathological observation of the cervical epithelium, micronucleus frequency in vaginal exfoliated cells and some biochemical parameters in the host liver. Quercetin was found to arrest or reverse the progression of cervical neoplasia. The micronucleus frequency was reduced following its administration. The potential anticarcinogenic effect of quercetin noted in this study is attributed to its antioxidant property which was reflected in the lipid peroxides and their role in the host detoxification system, as expressed in liver glutathione level, glutathione-S-transferase, glutathione peroxidase, catalase and superoxide dismutase activity. As an integral part of the diet quercetin may offer protection to the epithelium from the damaging effects of carcinogenic chemicals.

Keywords: quercetin; chemoprevention; 20-methylcholanthrene; micronucleus; antioxidant enzymes.

INTRODUCTION

Quercetin, a bioflavonoid, is a common non-nutrient component of many plants (Singleton, 1981) and an integral part of the human diet. Quercetin has been shown to have both mutagenic (MacGregor, 1900) and antimutagenic action (Malavielle *et al.*, 1996). This flavonoid is now considered to be an inhibitor of the carcinogenic process and a potential cancer chemopreventive agent (Makita *et al.*, 1996; Matsukawa *et al.*, 1997). No reports are available to date on the effect of this phytochemical during carcinogenesis of the uterine cervix.

Carcinoma of the uterine cervix can be developed by chemical induction in the mouse. This is a useful murine model for screening new chemopreventive agents, as during the carcinogenic process this model displays the various preneoplastic conditions characteristic in humans, dysplasia—mild, moderate and severe, which can be identified by cytological and histopathological studies.

The present report furnishes our observation on the protective effect of dietary quercetin on cervical epithelium during chronic exposure to a carcinogen, using cytological, histological and biochemical parameters.

MATERIALS AND METHODS

20-Methylcholanthrene (MC), quercetin, dithiobis(2-nitro)-benzoic acid (DTNB), 1-chloro-2-4-dinitrobenzene (CDNB), glutathione (GSH), glutathione reductase (GR), β-nicotinamide adenine dinucleotide phosphate, reduced form (β-NADPH), pyrogallol, diethylenetriamine penta acetic acid (DTPA) and thiobarbituric acid were purchased from Sigma Chemical Co., St Louis, MO, USA. Sodium dodecyl sulphate (SDS) was purchased from Gibco BRL, USA. Hydrogen peroxide solution (30%) was obtained from E. Merck (India). Hematoxylene, eosin and giemsa stains were obtained from Qualigens Fine Chemicals, India.

Cervical carcinoma was developed in 5–6 week old Swiss/Rb virgin female mice by delivering MC into the uterine cervix through the vaginal opening, at a dose of 10 mg/kg body weight daily for 30 days (Kehar and Wahi, 1967). One group received MC only. Another group received MC and quercetin at the minimal effective dose (2%) in the diet. Another group remained without MC but was fed quercetin. A normal control and a vehicle control group were also maintained. Vaginal exfoliated cells were collected at weekly intervals and smear preparations made for cytological observation, which was confirmed by histopathological study. No dysplasia was noted in the vehicle control group and so this group was discontinued after cytohistopathological observations. Micronucleus frequency was also examined in exfoliated cells by following the method of Schmid (1976) as modified by Vijayalaxmi and Rai (1996). All biochemical estimations were done on day 31 of the experiment.

GSH was estimated in the liver cytosolic fraction using DTNB by the method described by Sedlack and Lindsay (1968). GST activity was measured in the liver cytosol as expressed by the formation of CDNB–GSH conjugate following the method of Habig *et al.* (1974). Lipid peroxidation was estimated in the liver microsomal fraction by measuring the thiobarbituric acid reactive substances (TBARS) formed in the tissue using the method of Okhawa *et al.* (1979). Glutathione peroxidase (GPx) activity was determined in the postmitochondrial

* Correspondence to: Dr. S. Das, Department of Cancer Chemoprevention, Chittaranjan National Cancer Institute, Calcutta - 700 026, India.

Received 8 April 1999
Accepted 1 October 1999

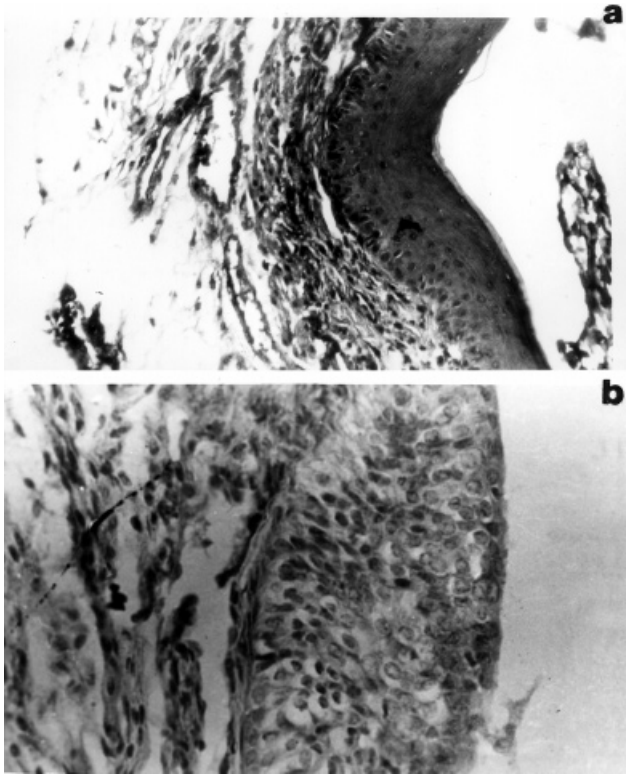

Figure 1. Histological observations: (a) normal uterine cervix, (b) carcinoma *in situ* following MC administration. Sections were stained with hematoxylene and eosin, magnification ×200.

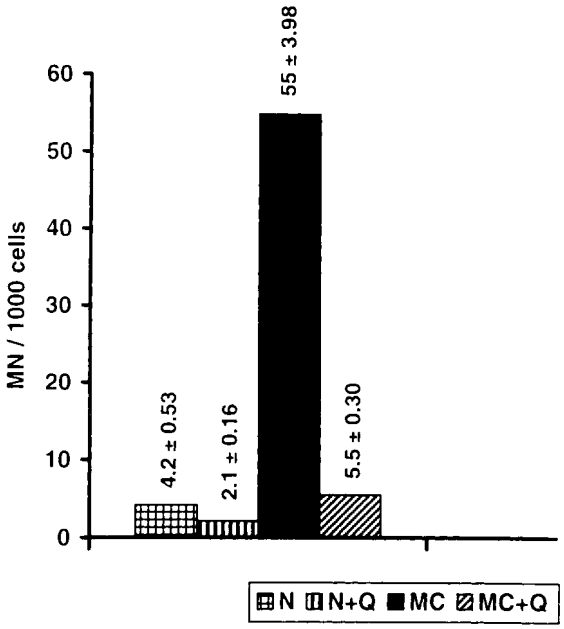

Figure 3. Effect of quercetin on micronucleus frequency. Micronucleus frequency (MN) was counted per 1000 exfoliated cells of uterine cervix in normal (N), normal + quercetin treated (N + Q), carcinogen treated (MC) and carcinogen + quercetin treated (MC + Q) mice. Data represent mean ± SE ($n = 8$).

fraction of the liver by the method of Paglia and Valentine (1967). Activity of catalase (CAT) was estimated by the method of Luck (1963) in the liver post nuclear homogenate. Superoxide dismutase (SOD) activity was determined by quantification of inhibition of pyrogallol autooxidation by the method of Marklund and Marklund (1974). Protein was estimated by Lowry's method (1951).

RESULTS

Cyto-histological observations

The characteristic cytological picture seen in vaginal exfoliated cells, revealing the normal 5 day oestrus cycle of prooestrus, oestrus, metaoestrus and dioestrus, was found to be disturbed following MC application. Histological observation revealed exposure to MC produced mild dysplasia in 65% after 7 days, of which 96% progressed to carcinoma *in situ* after 30 days through the stages of moderate and severe dysplasia (Fig. 1). In carcinogen treated animals which received dietary quercetin, mild dysplasia (Fig. 1). In carcinogen treated animals which received dietary quercetin, mild dysplasia was observed in only 34% cases after 14 days (Fig. 2). No further progression was noted within the experimental period of 1 month. Micronucleus frequency in the epithelial cells was significantly increased following carcinogen administration. Quercetin treatment could reduce the incidence of micronuclei (Fig. 3).

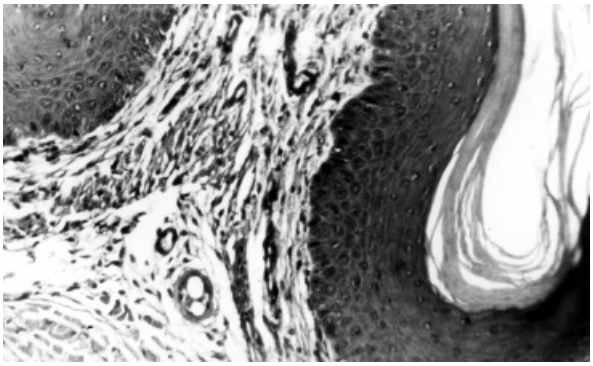

Figure 2. Mild dysplasia observed after quercetin treatment. For details see Fig. 1.

GSH level and GST activity

These were found to be decreased in the liver from those of normal values following exposure to the carcinogen. Quercetin treatment restored these deficiencies and significantly enhanced the level of GSH and the activity of GST above normal values observed in the present investigation. This effect was also noted in normal animals. These results are shown in Fig. 4.

Effect on lipid peroxidation

Considerable elevation of lipid peroxides was noted following MC administration. Oral intake of quercetin

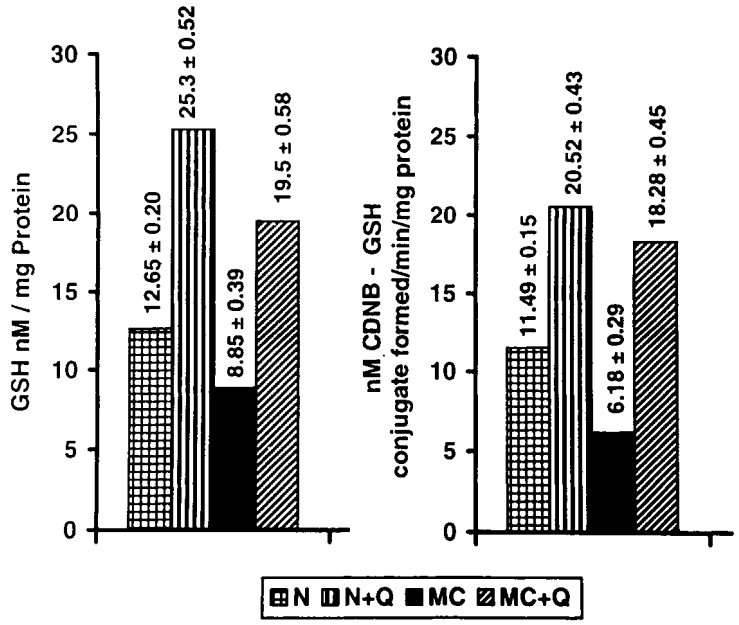

Figure 4. Effect of quercetin on GSH level and GST activity. Liver cytosolic fraction was examined in normal (N), normal + quercetin (N + Q), carcinogen treated (MC) and carcinogen + quercetin treated (MC + Q) mice to determine GSH level and GST activity. Data represent mean ± SE ($n = 8$).

significantly reduced lipid peroxidation both in normal and carcinogen treated groups (Fig. 5).

GPx, CAT and SOD activity

Chronic exposure to MC produced a significant decrease in GPx activity compared with normal animals. Concomitant treatment with quercetin was able to increase this activity. CAT activity, which was significantly increased in the carcinogen treated group, returned to the normal level following treatment with quercetin. However, quercetin feeding did not affect the CAT activity in the normal group. There was a decrease in SOD activity following MC administration. This also could be restored by quercetin treatment. All these results are depicted in Fig. 6.

DISCUSSION

Experimental studies had implicated a chemopreventive role of quercetin during carcinogenesis in the skin (Elangovan *et al.*, 1994), breast (Pereira *et al.*, 1996), colon (Matsukawa *et al.*, 1997) and oral cavity (Makita *et al.*, 1996), but this is perhaps the first report to demonstrate the influence of quercetin during the development of carcinoma in the uterine cervix. Histological evidence clearly indicated that quercetin arrested the progression of carcinogenesis by MC at the mild dysplasia stage.

Development of cancer involves genetic damage, which may be either induced or spontaneous due to exogenous and endogenous factors. The increased micronucleus frequency noted following chronic exposure of cervical epithelium to MC indicates damage to the genetic material in the epithelial cells. Quercetin could protect the cells from such damage as expressed by the reduced micronucleus frequency in the treated group. These observations suggest that quercetin, by

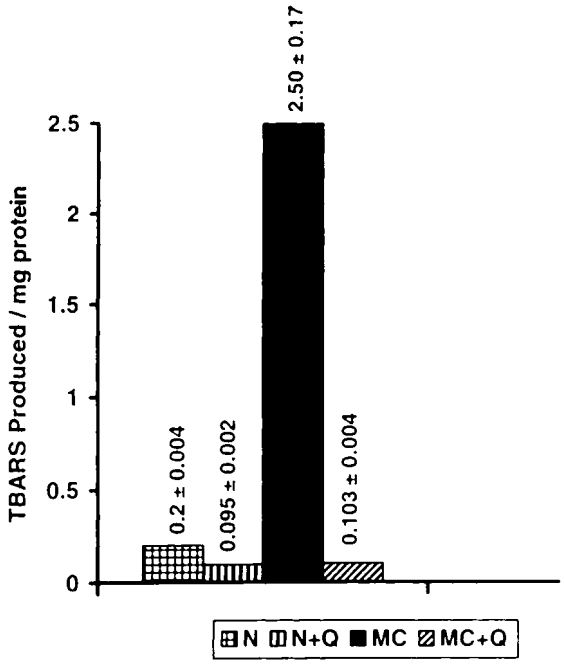

Figure 5. Effect of quercetin on lipid peroxidation. Estimation was made in liver microsomal fraction from normal (N), normal + quercetin (N + Q), carcinogen treated (MC) and carcinogen + quercetin treated (MC + Q) animals. Data represent mean ± SE ($n = 8$).

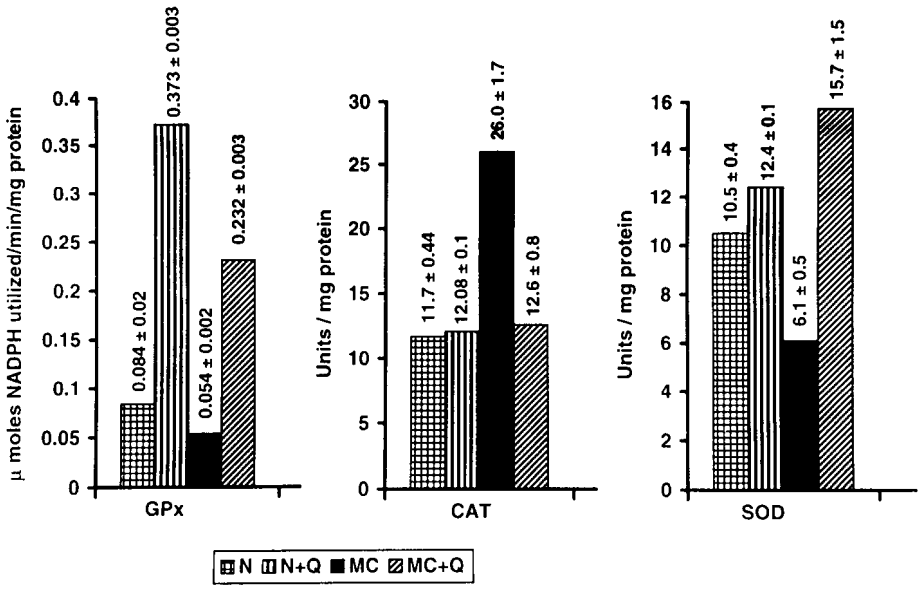

Figure 6. Effect of quercetin on antioxidant defence enzymes. GPX, CAT and SOD activity were measured in the livers of normal (N), normal + quercetin treated (N + Q), carcinogen treated (MC) and carcinogen + quercetin treated (MC + Q) animals. Data represent mean ± SE ($n = 8$).

virtue of its protective effect on DNA damage, helps significantly to prevent the process of carcinogenesis by MC.

GSH, the major free thiol present in all animal cells, participates in diverse biological processes, including detoxification of xenobiotics (Arias and Jakoby, 1976). GST plays an important role in initiating detoxification (Sedlack and Lindsay, 1968) by catalysing the conjugation of GSH to electrophilic foreign compounds for their elimination from the system. Reports on the association of GSH and GST in carcinogenesis and in cancer is often contradictory. Some reports indicate a decreased level of GSH in cervical neoplasia and invasive carcinoma (Kumar *et al.*, 1995; Basu *et al.*, 1991). Decreased plasma GSH was also noted in malignancies of breast, lung, liver, prostate and in lymphoma (Beuyer and Gilbert, 1985). On the other hand, it was seen that GSH content in the liver was increased following carcinogen administration and was in proportion to the carcinogenic potency (Meister and Griffith, 1979). Investigators also noted increased GST activity during malignancies (Tsuchida and Sato, 1992; Howie *et al.*, 1990; Peter *et al.*, 1992, 1990). The present study shows a reduction in liver GSH level and GST activity after 30 days of MC exposure, although in the initial stages we noted a sharp elevation. These results indicate that initially the host's defence system provides cellular protection by increasing the level of GSH and GST activity which help in neutralizing the xenobiotic. But chronic exposure to MC ultimately affects the defence mechanisms by significantly depleting the GSH level and GST activity which are unable to eliminate the carcinogen. Interestingly, oral administration of quercetin could increase the GSH level and GST activity both in normal and carcinogen treated animals (after 30 days MC treatment). This suggested that quercetin was capable of protecting the body even after 30 days of MC exposure. The quercetin-induced increase in the level of GSH and GST activity in normal animals showed the preventive activity of quercetin which can prepare the body to cope with damage.

Carcinogenesis is enhanced by lipid peroxides which also cause damage to cellular macromolecules by generation of reactive species. The MC-induced increase in lipid peroxides noted in our study could be significantly reduced by quercetin. This observation finds support from a report suggesting that quercetin can protect against lipid peroxidation (Chen *et al.*, 1996). Lipid peroxidation can result in the generation of various reactive oxygen species. Several enzymes are involved in the antioxidative defence system to protect cells and cellular DNA from damage by free oxygen radicals. To protect cells from damage radical and non-radical reactive oxygen species including peroxides and superoxides need to be inactivated enzymatically by CAT, SOD and GPx (Vang *et al.*, 1997). Decreased cellular activities of GPx and SOD were displayed in the MC treated group compared with normal, whereas the CAT activity was increased. Decreased GPx and SOD activity means that reactive species are accumulating in the body which ultimately cause oxidative damage to the cells and help the progression of carcinogenesis. Quercetin intake was found to significantly increase the GPx and SOD activity in both the MC treated and normal groups of animals. But no effect was noted on CAT activity. Increased GPx and SOD activity removes the peroxides and superoxides which are produced both in normal conditions and also in large amounts during carcinogen metabolism. Thus by increasing the GPx and SOD activity quercetin can prevent the accumulation of reactive species by trapping them.

From the present study it is quite clear that quercetin is capable of protecting the cervical epithelium from the damaging effect of the carcinogen possibly by increasing the body's defence mechanism. Therefore, this phyto-

chemical, which is abundantly present in many fruits and vegetables, should receive attention as a potential chemopreventive agent. Further studies are warranted with respect to determination of its toxicity and effective dose levels, so that it may be considered for prevention of human carcinomas.

Acknowledgements

The authors are grateful to Professor M. Siddiqi, Director, CNCI and Dr R. K. Bhattacharya, Emeritus Scientist (ICMR), CNCI for their expert comments and suggestions for preparation of the manuscript. Thanks are also due to Mr S. R. Datta for his excellent technical assistance.

REFERENCES

Arias, I. M., and Jakoby, W. B. (1976). *Glutathione: Metabolism and Functions*. Raven Press, New York.

Basu, J., Mikhail, M. S., Goldberg, G. L., Palan, P. R., and Romney, S. L. (1991). Measurement of ascorbic acid and glutathione in exfoliated cervico vaginal cells of smokers and women in cervical dysplasia. *Gynaecol. Obstet. Invest.* **30**, 48–51.

Beuyer, E., and Gilbert, T. (1985). Plasma glutathione in health and in patients with malignant diseases. *J. Lab. Clin. Med.* **105**, 581–584.

Chen, Z. Y., Chan, P. T., Ho, K. Y., Fung, K. P., and Wang, J. (1996). Antioxidant activity of natural flavonoids is governed by number and location of their aromatic hydroxyl groups. *Chem. Phys. Lipids.* **79**, 157–163.

Elangovan, V., Balasubramanian, S., Sekar, N., and Govindaswamy, S. (1994). Studies on the chemopreventive potential of some naturally occuring bioflavonoids in 7,12-dimethylbenz (a) anthracene induced carcinogenesis in mouse skin. *J. Clin. Biochem. Nutr.* **17**, 153–160.

Habig, W. H., Pabst, M. J., and Jakoby, W. B. (1974). Glutathione-S-transferase: the first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* **249**, 7130–7139.

Howie, A. F., Forrester, L. M., Glancey, M. J. *et al*. (1990). Glutathione-S-transferase and glutathione peroxidase expression in normal and tumour human tissues. *Carcinogenesis* **11**, 451–458.

Kehar, U., and Wahi, P. N. (1967). Cytologic and histologic behavior patterns of the premalignant lesions of the cervix in experimentally induced cervical dysplasia. *Acta Cytol.* **11**, 1–15.

Kumar, A., Sharma, S., Pundir, C. S., and Sharma, A. (1995). Decreased plasma glutathione in cancer of the uterine cervix. *Cancer Lett.* **94**, 107–111.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951). Protein measurement with Folin-phenol reagent. *J. Biol. Chem.* **193**, 265–275.

Luck, H. (1963). A spectrophotometric method for the estimation of catalase. In, *Methods of Enzymatic Analysis*, ed. by H. U. Bergmeyer, pp. 886. Academic Press, New York.

MacGregor, J. T. (1900). Genetic and carcinogenic effect of plant flavonoids: an overview. Nutritional and toxicology aspects of food safety. *Adv. Exp. Med. Biol.* **177**, 497–526.

Makita, H., Tanaka, T., Fujitsuka, H. *et al*. (1996). Chemoprevention of 4-nitroquinoline 1-oxide induced rat oral carcinogenesis by the dietary flavonoids chalone, 2-hydroxychalone and quercetin. *Cancer Res.* **56**, 4904–4909.

Malavielle, C., Hauteefeille, A., Pignatelli, B., Talaska, G., Vineis, P., and Bartsch, H. (1996). Dietary phenolics as antimutagens and inhibitors of tobacco related DNA adduction in urothelium of smokers. *Carcinogenesis* **17**, 2193–2200.

Marklund, S., and Marklund, G. (1974). Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur. J. Biochem.* **47**, 469–474.

Matsukawa, Y., Nishino, H., Okuyama, Y. *et al*. (1997). Effects of quercetin and/or restraint stress on formation of aberrant crypt foci induced by azoxymethane in rat colons. *Oncology* **54**, 118–121.

Meister, A., and Griffith, O. W. (1979). Effects of methionine sulfoximine analogs on the synthesis of glutamine and glutathione: possible chemotherapeutic implication. *Cancer Treatment Rep.* **63**, 1115–1121.

Okhawa, H., Ohishi, N., and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Annl. Biochem.* **95**, 351–358.

Paglia, D. E., and Valentine, W. N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* **70**, 158–169.

Pereira, M. A., Grubbs, C. J., Barnes, L. H. *et al*. (1996). Effects of the phytochemicals, curcumin and quercetin, upon azoxymethane-induced colon cancer and 7,12 dimethyl benzanthracene induced mammary cancer in rats. *Carcinogenesis* **17**, 1305–1311.

Peter, W. H. M., Boon, C. E. W., Roelofs, H. M. J., Wobbers, T. H., Nagengast, F. M., and Kremers, P. G. (1992). Expression of drug metabolizing enzymes and P-170 glycoprotein in colorectal carcinoma and normal mucosa. *Gastroenterology* **103**, 448–455.

Peter, W. H. M., Wormskamp, N. G. M., and Thies, E. (1990). Expression of glutathione-S-transferases in normal gastric mucosa and gastric tumours. *Carcinogenesis* **11**, 1593–1596.

Schmid, W. (1976). The micronucleus test for cytogenetic analysis. In, *Chemical Mutagens: Principles and Methods for their Detection*, ed. by A. Hollaender, pp. 62–86. Vol. **4**, Plenum Press, New York.

Sedlack, J., and Lindsay, R. N. (1968). Estimation of total protein bound and non-protein sulphydryl groups in tissues with Ellman reagent. *Anal. Biochem.* **25**, 192–205.

Singleton, V. L. (1981). Naturally occuring food toxicants: phenolic substances of plant origin common in risk foods. *Adv. Food Res.* **27**, 149–242.

Tsuchida, S., and Sato, K. (1992). Glutathione transferase and cancer. *Crit. Rev. Biochem. Mol. Biol.* **27**, 337–384.

Vang, O., Rasmussen, B. F., and Andersen, O. (1997). Combined effects of complex mixtures of potentially anti-carcinogenic compounds on antioxidant enzymes and carcinogen metabolizing enzymes in the rat. *Cancer Lett.* **114**, 283–286.

Vijayalaxmi, K. K., and Rai, S. P. (1996). Studies on the genotoxicity of tamoxifen citrate in mouse bone marrow cells. *Mutat. Res.* **368**, 109–114.

