Acta Pharmaceutica Sciencia
52: 23- 30 (2010)

Protective effect of curcumin against the genotoxic damage induced by tinidazole in cultured human lymphocytes

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Abstract

Tinidazole (TNZ) is used in the treatment of trichomoniasis, giardiasis and amebiasis. It has been reported as cytotoxic and genotoxic agent in various *in vitro* and *in vivo* models. Natural plant products having antioxidant properties can reduce the genotoxic effects of the drugs, if give in combination and thereby reduced the chances of developing cancer during the prolonged treatment with certain drugs. In the present study the effect of 5, 10 and 15 μM of curcumin was studied against the genotoxic doses of tinidazole i.e. 10 and 20 μg/ml, using lipid peroxidation, micronucleus and sister chromatid exchange as a parameters on cultured human lymphocytes. A significant dose dependent decrease in the genotoxic effects of TNZ was observed suggesting a protective role of curcumin against the drug.

Key words: Tinidazole, lipid peroxidation, micronucleus, sister chromatid exchange, curcumin.

Introduction

Tinidazole (TNZ) is an anti-parasitic drug widely used against protozoan infection in developed as well as developing countries. It has been approved for the treatment of trichomoniasis, giardiasis and amoebiasis by FDA (Sobel et al. 2001). TNZ has been reported as mutagenic in a strain sensitive to oxidative damage, *Salmonella typhimurium* tester strain TA 100 both with and without metabolic activation system, and negative in TA 98 (Dayan et al. 1982, Gupta et al. 1996). It has also been reported to induce micronuclei in mouse bone marrow cells *in vivo* (Leal Garja et al. 1984), chromosomal aberrations and sister chromatid exchanges (Lopez Nigro et al. 2003, Lopez Nigro et al. 2001, Lopez Nigro and Carballo 2008) and DNA single strand breaks in peripheral blood lymphocytes (Ferreiro et al. 2002). Curcumin (diferuloylmethane), the main yellow bioactive component of turmeric has been shown to have a wide spectrum of biological actions useful for human kind (Chattopadhyay et al. 2004). In the present study the effect of curcumin was studied on the genotoxic doses of TNZ using lipid peroxidation, sister chromatid exchange and micronucleus assay as a parameters.

Materials and Methods

Chemicals

Curcumin (Sigma); Tinidazole (Sigma); 1-methyl-2- phenylindole (Sigma); Acetonitrile (SRL, India); Methanol (Qualigens, India); HCl (Qualigens, India); TRIS (SRL, India); RPMI 1640,

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Phytohaemagglutinin-M, Antibiotic-antimycotic mixture (In vitirogen), Dimethyl sulphoxide (DMSO), Giemsa strain (Merck), Colchicine and 5-Bromodeoxyuridine (SRL), Cytochalasin-B (Sigma).

Human lymphocyte culture: Duplicate peripheral blood cultures were treated according to Carballo et al. (1993). Briefly, about 0.5 ml of heparinized blood samples were obtained from healthy female donor and were placed in a sterile glass culture tube containing 7 ml of RPMI-1640, supplemented with 1.5 ml of fetal calf serum, 0.1 ml of antibiotic-antimycotic mixture and 0.1 ml of phytohaemagglutinin. The culture tubes were placed in an incubator at 37°C for 24 h.

Lipid peroxidation assay: The present method of lipid peroxidation assay is based on the reaction of malonaldehyde with two molecules of 1-methyl-2-phenylindole at 45°C at 586 nm (Gerard-Monnier et al. 1985). The estimation of lipid peroxidation by Suryawanshi et al. (2006) was based on the same reaction. After 24 h, the treatments of tinidazole at final concentration of 1, 10, 20 µg/ml were given separately and kept for another 24 h at 37°C in an incubator. Simultaneously the activity of 10 and 20 µg/ml of tinidazole was examined in the presence of 5, 10 and 15 µM of curcumin separately and respectively to see the effect on the lipid peroxidation. Untreated was also run simultaneously. About 10 mM of 1-methyl-2-phenylindole was dissolved in 30 ml of acetonitrile and finally 10 ml of methanol was added to make 40 ml of volume. After incubation of 24 hr, the treated blood cultures were centrifuged at 3000 g for 20 min and the supernatant was collected. To a fresh tube about 1.3 ml of 10 mM 1-methyl-2-phenylindole (dissolved in acetonitrile) was taken in a microcentrifuge tube. 1 ml of supernatant was diluted 10 times with Tris buffer (20mM, pH 7.4) and 200 µl of diluted supernatant was taken from each of the treated culture along with the 200 µl of distilled water separately and vortexed. To each tube 300 µl of 37% HCl was added and vortexed. The tubes were incubated at 45°C for 40 min. After incubation the tubes were cooled on ice and then centrifuged at 15000 g for 10 min at 4°C and the readings were noted at 586 mm on a digital photo colorimeter (Metzer).

Cytokinesis – blocked micronucleus assay (CBMN): The presence of MN in a binucleated cell (BNC) was assayed by blocking the cells at the cytokinesis stage by the method of Fenech and Morley (1985). Lymphocyte culture was set as described earlier in the text. After 24 h, the treatment of tinidazole at final concentration of 1, 10 and 20 µg/ml was given separately and kept for another 48 h at 37°C in an incubator. Simultaneously the activity of 10 and 20 µg/ml of tinidazole was examined in the presence of 5, 10 and 15 µM of curcumin separately and respectively to see the effect on the micronucleus frequency. Untreated was also run simultaneously. Cyt-B (3 µg/5 ml culture) was added to the culture at 44 h after the initiation. After a total of 72 h incubation the cells were centrifuged at 800 g for 5 min. Supernatant was discarded and the cell pellets were treated with a hypotonic solution (0.0075M KCl). Cells were fixed with freshly prepared methanol: acetic acid (3:1) for 10 min and then centrifuged at approximately 800 g for 5 min. After air drying the slides were stained with 5% Giemsa stain in phosphate buffer (pH 6.8) for 10 min. BNCs surrounded by well-preserved cytoplasm were scored for the presence of MN. At least 2000 cytokinesis-blocked (CB) binucleated human lymphocytes with preserved cytoplasm were scored. MN was identified with the criteria followed by Fenech et al. (2003).

Sister chromatid exchange (SCE): For sister chromatid exchange analysis, bromodeoxyuridine (10 µg/ml) was added at the beginning of the culture. After 24 h, the treatments of tinidazole at 1, 10 and 20 µg/ml were given. Simultaneously, the treatments of 10 and 20 µg/ml of tinidazole were given separately and respectively with 5, 10 and 15 µM of curcumin. Mitotic arrest was done by adding, 0.2 ml of colchicine (0.2 µg/ml). Cells were centrifuged to 1000 rpm for 10 min. The supernatant was removed and 8 ml of prewarmed (37°C) 0.075 M kCl (hypotonic solution) was added. Cells were resuspended and incubated at 37°C for 15 min, and subsequently 5 ml of chilled fixative was added. The fixative was removed by centrifugation and the procedure was repeated twice. The slides were stained in 3% Giemsa

solution in phosphate buffer (pH 6.8) for 15 min. The sister chromatid exchange average was taken from an analysis of metaphase during second cycle of division (Perry and Wolff 1974).

Statistical analysis: Statistical analysis was performed by χ^2 test for micronucleus test and t- test was performed for lipid peroxidation and sister chromatid exchange using statistical soft Inc.

Results

Lipid peroxidation assay: The treatments of 1, 10 and 20 µg/ml of tinidazole (TNZ) were associated with a mean absorbance value of 0.05, 0.16 ($P<0.01$) and 0.24 ($P<0.01$) respectively (Table 1). The treatment of 10 µg/ml of TNZ with 5, 10 and 15 µM of curcumin was associated with 0.10 ($P<0.05$), 0.09 ($P<0.05$) and 0.08 ($P<0.05$) respectively. Similarly a dose dependent significant decrease was observed in the mean absorbance value with the treatment of 20 µg/ml of TNZ with 5 (0.14; $P<0.05$), 10 (0.12; $P<0.05$) and 15 µM (0.11; $P<0.05$) of curcumin (Table 1). The treatments of 5, 10 and 15µM of curcumin were associated with 0.03, 0.03 and 0.04 respectively (Table 1).

Table 1. Mean absorbance value at 586 nm after treatments for the estimation of lipid peroxidation

Treatments	Mean absorbance value (586 nm)
Tinidazole (µg/ml)	
1	0.05
10	0.16[a]
20	0.24[a]
Curcumin (µM) + Tinidazole (µg/ml)	
5 + 10	0.10[b]
10 + 10	0.09[b]
15 + 10	0.08[b]
5 + 20	0.14[b]
10 + 20	0.12[b]
15 + 20	0.11[b]
Curcumin (µM)	
5	0.03
10	0.03
15	0.04
Untreated	0.03

[a]$P < 0.01$ with respect to untreated.

[b]$P<0.05$ with respect to Tinidazole treatment.

Micronucleus assay: The treatments of 1, 10 and 20 µg/ml of tinidazole (TNZ) were associated with 11.5, 64 ($P<0.001$) and 70.5 ($P<0.001$) MN/10^3 respectively (Table 2). The treatment of 10 µg/ml of TNZ with 5, 10 and 15 µM of curcumin results in a dose dependent decrease in MN/10^3 cell i.e. 48 ($P<0.01$), 44 ($P<0.01$) and 37 ($P<0.01$) respectively (Table 2). Similarly, the treatment of 20 µg/ml of TNZ with 5, 10 and 15 µM of curcumin results in a dose dependent decrease in MN/10^3 cells i.e. 52.5 ($P<0.01$), 44.5 ($P<0.01$) and 37.5 ($P<0.01$) respectively.(Table 2). The treatments of 5, 10 and 15 µM of curcumin were associated with 10, 11 and 12 MN/10^3 cells respectively (Table 2).

Table 2. Effect of curcumin on tinidazole (TNZ) induced micronucleus on human peripheral blood lymphocytes

Treatments	BN Cells scored	Distribution of BN cells according to Number of MN				MN / 10^3 cells
		0	1	2	3	
Tinidazole (µg/ml)						
1	2000	1973	19	2	0	11.5
10	2000	1891	92	15	2	64[a]
20	2000	1882	98	17	3	70.5[a]
Curcumin (µM) + Tinidazole (µg/ml)						
5 + 10	2000	1912	80	8	0	48[b]
10 + 10	2000	1918	76	6	0	44[b]
15 + 10	2000	1928	70	2	0	37[b]
5 + 20	2000	1905	85	10	0	52.5[b]
10 + 20	2000	1918	75	7	0	44.5[b]
15 + 20	2000	1928	60	3	0	37.5[b]
Curcumin (µM)						
5	2000	1982	16	2	0	10
10	2000	1980	18	2	0	11
15	2000	1978	20	2	0	12
Untreated	2000	1984	15	1	0	8.5

[a]P < 0.01 (Significant as compared to untreated); [b]P<0.01 (Significant as compared to Tinidazole treatment)

Sister chromatid exchange analysis: The treatment of 1, 10 and 20 µg/ml of Tinidazole (TNZ) were associated with 2.42±0.42, 8.98±0.78 (P<0/01) and 12.42±0.92 (P<0.01) SCE/cell respectively (Table 3). The treatment of 10 µg/ml of TNZ with 5, 10 and 15 µM of curcumin results in a dose dependent decrease in SCEs/cell i.e. 4.32±0.58 (P<0.05), 4.12±0.52 (P<0.05) and 3.42±0.48 (P<0.05) respectively (Table 3).

Table 3. Sister chromatid exchanges after tinidazole (TNZ) and curcumin treatment.

Treatments	Cells scored	SCE / Cell ± SE
Tinidazole (µg/ml)		
1	50	2.42 ± 0.42
10	50	8.98 ± 0.78[a]
20	50	12.42 ± 0.92[a]
Curcumin (µM) + Tinidazole (µg/ml)		
5 + 10	50	4.32 ± 0.58[b]
10 + 10	50	4.12 ± 0.52[b]
15 + 10	50	3.42 ± 0.46[b]
5 + 20	50	8.34 ± 0.74[b]
10 + 20	50	7.22 ± 0.68[b]
15 + 20	50	7.02 ± 0.64[b]
Curcumin (µM)		
5	50	1.72 ± 0.32
10	50	1.74 ± 0.34
15	50	1.78 ± 0.38
Untreated	50	1.54 ± 0.20

[a]P < 0.01 (Significant as compared to untreated)

[b]P<0.05 (Significant as compared to Tinidazole treatment)

Similarly, the treatments of 20 μg/ml of TNZ with 5, 10 and 15 μM of curcumin results in a dose dependent significant decrease in SCEs/cell i.e. 8.34±0.74 ($P<0.05$), 7.22±0.68 ($P<0.05$) and 7.02 ± 0.64 ($P<0.05$) respectively. The treatment of 5, 10 and 15 μM of curcumin were associated with 1.72 ± 0.32, 1.74 ± 0.34 and 1.78 ± 0.38 SCE/cell respectively (Table 3).

Discussion

The results of the present study show that the curcumin reduced the lipid peroxidation and genotoxic damage induced by tinidazole. The reduction of the nitro group and generation of short-lived reactive intermediates are the basis of tinidazole parasitidal activity (Ferreiro et al. 2002). TNZ is reduced by ferredoxin mediated electron transport system (Fung and Doan, 2005). These short lived intermediates may covalently bind to DNA, resulting in its damage (Tasca et al. 2003), and may also results in the peroxidation of lipids i.e. phospholipids of biological membranes (Halliwell and Aruoma 1991). It is known that TNZ is mainly metabolized by CYP3A4, which is expressed in human T-lymphocytes and macrophages (Nagai et al. 2002).

In the present study we have estimated the lipid peroxidation in terms of quantifying malondialdehyde (MDA). Lipid peroxidation products of polyunsaturated fatty acids (PUFAs) are considered of importance for genotoxic effects (Comporti, 1989; Eder et al. 2004). Reactive oxygen species can induce lipid peroxidation and per oxidative fatty-acid fragments and radicals can *vice versa* lead to the formation of reactive oxygen species (Eder et al. 2004). Malondialdehyde (MDA) and 4-hydroxy-2-noneal (HNE) are the two most prominent lipid peroxidative products (Esterbauer et al. 1991). The method we used established for lipid peroxidative assay in present study gives negligible reaction with 4-hydroxynonenal. In the presence of hydrochloric acid malondialdehyde reacts with two molecules of 1-methyl-2-phenylindole to yield a stable chromophore with intense maximal absorbance at 586 nm (Gerard-Monniere et al. 1985). The assay performed in hydrochloric acid based medium enables the specific measurement of MDA in the presence of 4-hydroxyalkenals, because in the presence of hydrochloric acid, 4-hydroxyalkenals undergo an irreversible cyclization reaction (Gerard-Monnier et al. 1985). Ion radicals generated during oxidative stress lead to a chain reaction called lipid peroxidatioin. Aldehydes generated from lipid peroxidation form DNA adducts and lipid hydroperoxides (LOOH) results in extensive single and double strand breaks (Devipriya et al. 2008). Various constituents of fruits and vegetables possess antioxidant activity that protects the membranes during the oxidative stress (Akrishnan et al. 2001, Prasad et al. 2006, Siddique and Afzal in press). An increase in the genotoxic damage is associated with an increased overall risk of cancer (Hagmar 1994, 1998). The micronucleus is a well-known marker of genotoxicity and any reduction in the frequency of the genotoxic endpoint gives an indication of the antigenotoxicity of a particular compound (Albertini et al. 2000). The results of the present study showed that the curcumin is potent enough to reduce the genotoxic damage of tinidazole thereby reducing the chances of developing cancers. The high concentration/ prolonged use of a certain drugs may leads to the cancer induction in animals due to the DNA damage. Sister chromatid exchange (SCE) is a more sensitive indicator of genotoxic effects (Tucker and Preston 1996). There is an excellent correlation between carcinogenicity and SCE inducing ability of large number of chemicals (Gebhart 1981).

Natural plant products have been reported to reduce the genotoxic effects of steroidal and anti-cancerous drugs (Siddique et al. 2006, Siddique and Afzal 2004, 2005, 2008). Curcumin is a well-known antioxidant (Chattopadhyay et al. 2004). The antioxidant mechanism of curcumin is attributed to its unique conjugated structure, which includes two methoxylated phenols (Masuda et al. 2001). Curcumin inhibits the generation of reactive oxygen species (ROS) like superoxide anions, H_2O_2 and nitrite radical generation by activated macrophages, that play an important role in inflammation and also in the genotoxicity (Joe et al. 1994). The reduction in the genotoxic damage by curcumin is attributed to its free radical scavenging antioxidant property. The reduction in the lipid peroxidation is also concurrent with the other studies performed using curcumin (Kamal-Eldin et al. 2000, Began et al. 2001). Curcumin also has antiprotozoal activity (Koide et al. 2002). In our present study with tinidazole curcumin reduced the genotoxic effects of the drug. The results suggest that curcumin can be supplemented during the treatment with tinidazole. It will not only decrease the possible chances of DNA damage/lipid peroxidation but will also contribute to the antiprotozoal activity.

Acknowledgements

Thanks are due to the DST, New Delhi for the project No: SR/FT/LS-003/2007 under the Fast Track Scheme for Young Scientist to the author (Y.H.S.) and to the Chairman, Department of Zoology, A.M.U., Aligarh for laboratory facilities.

References

Akrishnan, V.P. and Menon, V.P. (2001). Potential, role of antioxidants during ethanol-induced changes in the fatty acid composition and arachidonic acid metabolites in male wistar rats. *Cell Biol. Toxicol.* 17: 11-22.

Albertini, R.J., Anderson, D., Douglas, G.R., Hagmar, L., Heminiki, K., Merelo, F., Natarajan, A.T., Norppa, H., Shuker, D.E.G., Tice, R., Walters, N.D. and Aitio, A. (2000). IPCS guideline for the monitoring of genotoxic effects of carcinogens in humans. *Mutat. Res.* 463: 111-172.

Began, G., Sudharshan, E., Udayashankar, K. and Appu Rao, A.G. (1999). Interaction of curcumin with phosphatidylcholine: a spectrofluorometric study. *J. Agr. Food Chem.* 47: 4992-4997.

Carballo, M.A., Alvarez, S. and Boveris, A. (1993). Cellular stress by light and Rose Bengal in human lymphocytes. *Mutat. Res.* 288: 215-222.

Chattopadhyay, I., Biswas, K., Bandyopadhyay, U. and Banerjee, R.K. (2004). Turmeric and curcumin: Biological actions and medicinal applications. *Curr. Sci.* 87: 44-53.

Comporti, M. (1989). Three models of free radical-induced cell injury. *Chem. Biol. Interact.* 72: 1-56.

Dayan, J., Grajer, M.C. and Deguingand, S. (1982). Mutagenic activity of 4 active-principle forms of pharmaceutical drugs. Comparative study in the *Salmonella thyphimurium microsome* test and the HGPRT and Na^+/K^+ ATPase systems in cultured mammalian cells. *Mutat. Res.* 102: 1-12.

Devipriya, N., Sudheer, A.R., Srinivasan, M. and Menon, V.P. (2008). Quercetin ameliorates gamma radiation induced DNA damage and biochemical changes in human peripheral blood lymphocytes. *Mutat. Res.* 654: 1-7.

Eder, E., Wacker, M. and Wanek, P. (2004). Lipid peroxidation-related 1, N^2-propandodeoxyguanosine-DNA adducts induced by endogenously formed 4-hydroxy-2-noneal in organs of female rats fed diets supplemented with sunflower, rapeseed live or coconut oil. *Mutat. Res.* 654: 101-107.

Esterbauer, H., Schaur, R.J. and Zollner, H. (1991). Chemistry and biochemistry of 4-hydroxynoneal, malonaldehyde and related aldehydes. *Free Rad. Biol. Med.* 11: 81-128.

Fenech, M., Chang, W.P., Krisch-Volders, M., Holland, S.N., Bonassi, S. and Zeiger, E. (2003). Human project: detailed description of the scoring criteria for the cytokinesis – block micronucleus assay using isolated human lymphocyte cultures. *Mutat. Res.* 534: 65-75.

Fenech, M. and Morley, A.A. (1985). Measurement of micronuclei in lymphocytes. *Mutat. Res.* 147: 29-36.

Ferreiro, G.R., Badias, L.C., Lopez Nigro, M., Palerno, A., Murdy, M., Gozalez Elio, P. and Carballo, M.A. (2002). DNA single strands breaks in peripheral blood lymphocytes induced by three nitroimidazole derivatives. *Toxicol. Lett.* 132: 109-115.

Fung, H.B. and Doan, T.L. (2005). Tinidazole: a nitroimidazole antiprotozoal agent. *Clin. Ther.* 27: 1859-1884.

Gebhart, E. (1981). Sister chromatid exchange (SCE) and structural chromosomes aberrations in mutagenicity testing. *Hum. Genet.* 58: 235-254.

Gerard-Monnier, D., Erdelmeier, I., Regnard, K., Mozehenry, N., Yadan, J.C. and Chaudierel, J. (1985). Reactions of 1-methyl-2-phenylindole with malondialdehyde and 4-hydroxyalkenals Analytical applications to a colorimetric assay of lipid peroxidation. *Chem. Res. Toxicol.* 11: 1175-1183.

Gupta, R.L., Vats, V. and Juneja, T.R. (1996). Activation of tinidazole, an antiprotozoal drug to a mutagen by mammalian liver S9. *Mutat. Res.* 370: 195-201.

Hagmar, L., Bonassi, S., Stromberg, U., Brogger, A., Knudson, J.E., Norppa, H. and Reuterwall, C. (1998). Chromosomal aberration in human lymphocytes predict human cancer: a report from the European study Group on Cytogenetic Biomarkers and Health (ESCH). *Cancer Res.* 58: 4117-4121.

Hagmar, L., Brogger, A., Hansteen, I.L., Heins, S., Hoggstedt, B., Knudsen, L., Lambert, B., Linmaimaa, K., Mitelman, F., Nordenson, I., Reuterwall, C., Salomaa, S., Skerfuing, S. and Sorsa, M. (1994). Cancer risk in humans predicted by increased level of chromosomal aberrations in human lymphocytes: Nordic study Group on the Health Risk of Chromosome Damage. *Cancer Res.* 54: 2919-2922.

Halliwell, B. and Aruoma, O.L. (1991). DNA damage by oxygen-derived species *FEBS* 281: 9-19.

Joe, B. and Lokesh, B.R. (1994). Role of capsaicin, curcumin and dietary M-3 fatty acids in lowering the generation of reactive oxygen species in rat peritoneal macrophages. *Biochem. Biophysics Acta* 1224: 255-263.

Kamal-Eldin, A., Frank, J., Razdan, A., Tengbald, S., Basu, S. and Vessby, B. (2000). Effects of dietary phenolic compounds on tocopherol, cholesterol, and fatty acids in rats. *Lipids* 35: 427-435.

Koide, T., Nose, M., Ogihara, Y., Yabu, Y. and Ohta, N. (2002). Leishmanicidal effect of curcumin *in vitro*. *Biol. Pharmacol. Bull.* 25: 131-133.

Leal Garza, C., Montes de Oca, R., Baca, S. and Garza Chapa, R. (1984). Tinidazole and emenitine cytogenetic effects evaluated by the micronucleus test in mice. *Arch. Investig. Med. (Mex)* 15: 311-316.

Lopez Nigro, M.M. and Carballo, M.A. (2008). Genotoxicity and cell death induced by tinidazole (TNZ). *Toxicol. Lett.* 180: 46-52.

Lopez Nigro, M.M., Gadano, A.B. and Carballo, M.A. (2001). Evaluation of genetic damage induced by a nitronidazole derivative in human lymphocytes: tinidazole (TNZ). *Toxicol. in vitro* 15: 209-213.

Lopez Nigro, M.M., Palermo, A.M., Murdy, M.D. and Carballo, M.A. (2003). Cytogenetic evaluation of two nitroimidazole derivatives. *Toxicol. in vitro* 17: 35-40.

Masuda, T., Maekawa, T., Hidaka, K., Bando, H., Takeda, Y. and Yamaguchi, H. (2001). Chemical studies an antioxidant mechanisms of curcumin: analysis of oxidative coupling products from curcumin and linoleate. *J. Agr. Food Chem.* 49: 2539-2547.

Nagai, F., Hiyoshi, Y., Sugimachi, K. and Tamura, H. (2002). Cytochrome P450 (YP) expression in human myeloblastic and lymphoid cell lines. *Biol. Pharmacol. Bull.* 25: 383-385.

Perry, P. and Wolff, S. (1974). New Giemsa method for the differential staining of sister chromatids. *Nature* 151: 156-158.

Prasad, N.R., Srinivasan, M., Pugalendi, K.V. and Menon, V.P. (2006). Protective effect of ferfulkic acid on γ-radiation induced micronuclei, dicentric aberration and lipid peroxidation in human lymphocytes. *Mutat. Res.* 603: 129-134.

Siddique Y.H., Beg, T. and Afzal, M. Protective effect of ascorbic acid against oxidative damage induced by hydrogen peroxide in cultured human peripheral blood lymphoytes. *Indian J. Clin. Biochem.* (in press)

Siddique, Y.H. and Afzal, M. (2004). Antigenotoxic effects of allicin against SCEs induced by methyl methane sulphonate in cultured mammalian cells. *Indian J. Exp. Biol.* 42: 437-438.

Siddique, Y.H. and Afzal, M. (2005). Protective role of allicin and L-ascorbic acid against the genotoxic damage induced by chlormadinone acetate in cultured human lymphocytes. *Indian J. Exp. Biol.* 43: 769-772.

Siddique, Y.H., Beg, T. and Afzal, M. (2006). Protective effect of nordihydroguaiaretic acid (NDGA) against norgestrel induced genotoxic damage. *Toxicol. in vitro* 20: 227-233.

Siddique, Y.H., Beg, T. and Afzal, M. (2008). Antigenotoxic effect of apigenin against anti-cancerous drugs. *Toxicol. in vitro* 22: 625-631.

Sobel, J.D., Nyirjesy, P. and Brown, W. (2001). Tinidazole therapy for metronidazole-resistant vaginal trichomoniasis. *Clin. Inf. Dis.* 33: 1341-1346.

Suryawanshi, N.P., Bhutey, A.K., Nagdeote, A.N., Jadhav, A.A. and Manoorkar, G.S. (2006). Study of lipid peroxide and lipid profile in diabetes mellitus. *Indian J. Clin. Biochem.* 27: 126-130.

Tasca, T., Borges, F.P., Bonan, C.D., De Carli, G.A., Batastini, A.M. and Sarkis, I.I. (2003). Effects of metronidazole and tinidazole on NTPDase 1 and ecto-5'-nucleotidase from intact cells of *Trichomonas vagimalis*. *FEMS Microbiol. Lett.* 226: 379-384.

Tucker, J.D. and Preston, R.J. (1996). Chromosomes aberrations, micronuclei, aneuploidy, sister chromatid exchanges and cancer risk assessment. *Mutat. Res.* 365:147-159.

Received: 30.05.2009
Accepted: 01.10.2009