Journal of BUON **14:** 647-652, 2009

ORIGINAL ARTICLE

Effects of vitamin C and n-acetylcysteine against cyclophosphamide-induced genotoxicity in exfoliated bladder cells of mice *in vivo*

N. Gurbuz[1], A. Ozkul[2], S. Burgaz[3]

[1]Laboratory of Animal Breeding and Experimental Research Center, Faculty of Medicine, Gazi University, Besevler, Ankara; [2]Department of Pathology, Faculty of Veterinary, University of Ankara, Aydinlikevler, Ankara; [3]Department of Toxicology, Faculty of Pharmacy, Gazi University, Hipodrom, Ankara, Turkey

Summary

Purpose: *To investigate the effects of vitamin C and N-acetylcysteine (NAC) against cyclophosphamide (CP) –induced genotoxic damage in exfoliated bladder cells of mice by micronucleus (MN) assay.*

Methods: *For each experimental step, 6-8 Swiss albino balb/c male mice were used. CP was used as positive control. Vitamin C (10, 30 and 60 mg/kg) and CP (51.6 mg/kg) were administered intraperitoneally to the experimental animals. Vitamin C was administered twice, one dose 24 h prior to the CP administration and the second dose simultaneously with the CP. NAC (200, 400 and 800 mg/kg) was administered by gavage for 7 consecutive days before the injection of CP. Distilled water and normal saline as negative controls I and II were used, respectively. Ten days after CP treatment, the mice were sacrificed and bladders were isolated and cut, and exfoliated cells were scraped from the bladder walls. Air-dried smears were stained by Feulgen reaction. MN frequencies were scored in 1000 epithelial cells per animal and defined as MN per thousand (‰).*

Results: *Three doses of vitamin C (10, 30 and 60 mg/kg) showed a significant inhibitory effect on MN frequencies in mouse bladder cells when compared with those of positive control group ($p < 0.05$). Dose-dependent inhibitory effect of vitamin C was observed only between the doses of 10 and 60 mg/kg ($p < 0.05$). Histopathological changes that depended on CP- induced inflammatory infiltration and haemorrhage in mucosa propria were not observed in all 3 vitamin C doses. Three doses of NAC (200, 400 and 800 mg/kg) inhibited the CP-induced genotoxicity ($p < 0.05$), however, the antigenotoxic effect of NAC was not dose-dependent. Histopathological changes that depended on CP-induced inflammatory infiltration and haemorrhage in mucosa propria were not observed in 200 and 400 mg/kg NAC dosage. The extent of desquamation in bladder was similar in all 3 doses of NAC when compared with the positive control group.*

Conclusion: *Our study indicated that vitamin C and NAC reduced the CP-induced MN frequencies in target (bladder) cells of mice by 41-71% in all cases. The modifying effects of vitamin C and NAC against CP-induced genotoxic damage may be due to their antioxidant, nucleophilic properties and to the ability to act as precursors of glutathione.*

Key words: chemoprevention, exfoliated bladder cells, genotoxicity, micronucleus, N-acetylcysteine, vitamin C

Introduction

Chemoprevention is a strategy used to halt the development of cancers in humans by administering one or more chemicals, either as drugs or as natural dietary constituents. Thus there is a need to use biomarkers that may serve as a means to detect exposure to carcinogenic influences, as prognostic or diagnostic indicators, or as intermediate endpoints in intervention studies [1].

Approximately 92% of all of human malignancies occur in epithelial tissues. The MN assay in exfoliated cells is an innovative genotoxicity technique which holds promise for the study of epithelial carcinogens [2]. Chemopreventive studies have demonstrated that alterations in MN frequencies can provide early indication of the protective effect of a chemopreventive agent in a target tissue [3].

The evaluation of genotoxic damage in exfoliated cells from the bladder of mice might be useful to get information about antimutagenesis and anticarcinogen-

Correspondence to: Neslihan Gurbuz, PhD. Gazi University Faculty of Medicine, Laboratory of Animals Breeding and Experimental Research Center, 06500 Besevler, Ankara, Turkey. E-mail: neslihan.gurbuz@gazi.edu.tr

Received 19-01-2009; Accepted 27-02-2009

esis studies [4,5].

Vitamin C and NAC inhibit mutations and cancer in a variety of experimental test systems and are thus considered to be among the most promising cancer chemopreventive agents [6,7]. Konapacka et al. [5] have shown that vitamins C, E and beta carotene influence the level of radiation-induced damage in exfoliated bladder cells of mice.

To our knowledge, there is no study on the modifying effect of vitamin C and NAC against CP-induced genotoxicity in exfoliated bladder cells of mice.

The present study was undertaken to obtain information about the possible effects of vitamin C and NAC against CP-induced genotoxic damage in exfoliated bladder cells of mice using the MN assay.

Methods

Animals

Swiss-albino balb/c male mice used in this experiment were 8-10 weeks old and weighing 20-30 g. The animals were obtained from Gulhane Military School, Laboratory of Animals Department. The mice were maintained under proper environmental conditions, i.e. temperature 25±2° C, and humidity at ~50% with a 12 h light/dark period. All animals received a standard diet.

Chemicals (doses, treatment and sampling)

Cyclophosphamide (CAS No. 6055-19-2; Endoxan-Asta, 1 g in saline, Asta Med. Co.) was used as positive control. Vitamin C (L-Ascorbic Acid, CAS No. 50-81-7; Extra pure, Merck KGaA, Cat. No. 500069, Darmstadt, Germany) was administered in 3 different doses (10, 30, 60 mg/kg) to assess its antigenotoxic effects [8]. NAC (CAS No. 616-91-1; Lot: 2100389776: 100%, Bilim Drug Co., Turkey) was given in 3 different doses (200, 400, 800 mg/kg) for the evaluation of its antigenotoxic effects [9].

Since no clear information exists over the turnover time of mouse bladder epithelial cells, the maximum response time of CP and dose-response relationship for this time were also determined. Optimal sampling time was selected on the basis of our preliminary experiments that included scraping of exfoliated cells from the bladder of mice at 2,5,7,10,15 and 17 days after treatment with 51.6 mg/kg of CP and also taking into consideration the results of Konapacka [4]. This time was the 10th day after treatment with 51.6 mg/kg of CP. At the same time histopathologic changes such as extent of desquamation, hydropic degeneration, oedema, inflammatory infiltration, and haemorrhage in mucosa were also recorded for the selection of optimal time and dose of CP and for assessment of the antigenotoxic effects of different doses of vitamin C and NAC.

Animal groups

Swiss albino balb/c mice were divided into 9 groups consisting of 6-8 animals each. NAC, vitamin C and CP (positive control) were prepared in bidistilled water. Vitamin C and CP were administered intraperitoneally to experimental animals. Vitamin C was administered twice, one dose 24 h prior to the CP administration and the second dose simultaneously with CP administration. NAC was administered by gavage for 7 consecutive days before the injection of CP. The volume of all administered compounds was 0.1-0.5 ml. The control group consisted of animals given only distilled water (negative control I) and normal saline (negative control II) either intraperitoneally or by gavage. All animals were sacrificed at the end of the experiment, and body and liver weight were measured.

Exfoliated bladder cell MN analysis and scoring

Ten days after CP treatment, the mice were sacrificed and bladders isolated. The bladders were cut and exfoliated cells were scraped from the bladder walls. Air-dried smears were stained by a modified Feulgen reaction [4]. MN frequencies were scored in 1000 epithelial cells per animal and defined as MN per thousand (‰). Criteria for identifying MN were based on those given by Countryman et al [10]. All of the slides were scored by the same observer.

Histopathological examination

Tissue sections were fixed in 10% buffered neutral formalin and processed by conventional methods embedded in parafin wax, cut at 5 μ, stained with hematoxylin-eosin [13] and investigated for histopathological changes such as extent of desquamation, hydropic degeneration, oedema, inflammatory infiltration and hemorrhage in mucosa.

Statistical analysis

The results were analyzed for significant differences using the Mann-Whitney test at $p < 0.05$.

Results

MN frequencies in exfoliated urinary bladder

cells of mice treated with different doses of vitamin C are given in Table 1.

All the 3 doses of vitamin C showed a significant inhibitory effect on MN frequencies in mouse bladder cells when compared with those of positive control group ($p < 0.05$, Table 1). Although MN frequencies tended to decrease with increasing doses, dose-dependent inhibitory effect of vitamin C was only observed between 10 and 60 mg/kg ($p < 0.05$, Table 1).

The 3 doses of vitamin C, when given alone, did not produce any genotoxic effect when compared with the negative control ($p > 0.05$).

No significant differences in body, liver and relative liver weights were observed after treatment of different doses of vitamin C + 51.6 mg/kg CP compared to the negative control II ($p > 0.05$), except 30 mg/kg vitamin C + 51.6 mg/kg CP dose group where a significant difference ($p < 0.05$) was noted between positive and negative control I groups (data not shown).

Histopathological changes that could depend on CP-induced inflammatory infiltration and hemorrhage in mucosa were not observed in all 3 doses of vitamin C.

MN frequencies in exfoliated urinary bladder cells of mice treated with different doses of NAC are shown in Table 2.

All 3 doses of NAC showed a significant inhibitory effect on MN frequencies in mouse bladder cells when compared with those of the positive control group ($p<0.05$, Table 2). Although there was a trend towards decreasing MN frequencies with increasing doses, MN frequency in bladder cells was not dose-dependent ($p>0.05$, Table 2).

No significant differences in body, liver and relative liver weights were observed after treatment with different doses of NAC + 51.6 mg/kg CP compared to the negative control group II ($p>0.05$), except 800 mg/kg NAC + 51.6 mg/kg CP dose group where a significant difference ($p<0.05$) was noted between positive and negative control I group (data not shown).

Histopathological changes that could depend on CP-induced inflammatory infiltration and hemorrhage in mucosa were not observed in doses of 200 and 400 mg/kg NAC. The extent of desquamation in bladder was similar in all 3 does of NAC when compared with the positive control (CP) group.

Table 1. MN frequencies in exfoliated urinary bladder cells of mice treated with different doses of vitamin C

Groups	*n*	*MN frequencies (‰) Mean ± SD*	*Inhibition (%)*
Negative control I*	8	3.38±1.69	
Negative control II **	6	3.66±1.03	
1 Vitamin C 10 mg/kg	7	3.57±1.27	
2 Vitamin C 30 mg/kg	7	2.43±0.97	
3 Vitamin C 60 mg/kg	7	3.43±0.97	
Positive control[a]	8	16.5±3.55	
4 Vitamin C 10 mg/kg+CP	6	9.71±1.50[b,a,*,**]	41
5 Vitamin C 30 mg/kg+CP	8	8.13±2.90[b,a,*,**]	51
6 Vitamin C 60 mg/kg+CP	8	7.00±2.27[b,c,a,*,**]	58

CP: cyclophosphamide, MN: micronucleus
[a]$p < 0.05$ compared with positive control; CP 51.6 mg/kg
[b]$p < 0.05$ compared with 10,30,60 mg/kg vitamin C
[c]$p < 0.05$ compared with 10 mg/kg vitamin C+ CP
*$p < 0.05$ compared with negative control I; bidistilled water
**$p < 0.05$ compared with negative control II; normal saline

Table 2. MN frequencies in exfoliated urinary bladder cells of mice treated with different doses of n-acetylcysteine

Groups	*n*	*MN frequencies (‰) Mean ± SD*	*Inhibition (%)*
Negative control I*	8	2.63±0.91	
Negative control II**	7	2.71±1.38	
1 NAC 200 mg/kg	6	2.83±1.17	
2 NAC 400 mg/kg	6	2.33±1.21	
3 NAC 800 mg/kg	6	2.00±0.89	
Positive control[a]	6	17.0±3.09	
4 NAC 200 mg/kg+CP	6	7.66±2.94[b,a,*,**]	55
5 NAC 400 mg/kg+CP	8	8.39±3.54[b,a,*,**]	51
6 NAC 800 mg/kg+CP	6	5.00±1.41[b,a,*,**]	70

CP: cyclophosphamide, NAC: n-acetylcysteine, MN: micronucleus
[a]$p < 0.05$ compared with positive control; CP 5.16 mg/kg
[b]$p < 0.05$ compared with 200, 400, 800 mg/kg NAC
*$p < 0.05$ compared with negative control I; bidistilled water
**$p < 0.05$ compared with negative control II; normal saline

Discussion

The chemopreventive effect of vitamin C and NAC against CP-induced genotoxicity in exfoliated bladder cells of mice has been studied in this article.

Some epidemiological and experimental studies have proved that natural substances in food and synthetic chemicals possess protective effects against environmental mutagens and carcinogens [12].

The MN assay is one of the most widely applied short -term tests used in genotoxicity studies and has become one of the most important tests implemented in the evaluation of mutagenicity and carcinogenicity [13].

MN assay is applied to determine genotoxicity in oral cavity, lung, spleen, urinary bladder, testis, cervix, vagina, esophagus, corneal epithelial and hair follicle cells [13-17]. When the target tissue is epithelial tissue, the exfoliated cell MN assay has advantages over the more widely used MN assay in lymphocytes. The target tissue can be studied directly without stimulation, whereas lymphocytes must first be stimulated. The re-

lationship in epithelial tissues between short-term assay of cytogenetic effects and long-term assay of carcinogenic effects may also be investigated [13]. The determination of MN frequencies in exfoliated epithelial cells may not only indicate the genotoxic exposures but it evaluates the predictive value of this model for the detection of human cancer risks [13]. So, it is probable that studies conducted in several target tissues in animal models to evaluate the chemopreventive effects of chemicals may add some important data both in anticarcinogenesis/mutagenesis studies of experimental animals and human populations.

In this paper, CP is used as a model mutagen to screen the genotoxic damage in exfoliated bladder cells of mice.

It is well-known that CP undergoes enzymatic and nonenzymatic metabolic activation to produce its metabolites acrolein and phosphoramide mustard (PM). PM is responsible for the antineoplastic, clastogenic and mutagenic properties of CP [18]. Acrolein is a highly reactive compound that induces DNA single-strand breaks, contributing eventually to the cytotoxicity and mutagenicity of CP [19,20]. Therefore, CP has been used frequently as a model mutagen in several experimental studies to investigate anticlastogenic and antimutagenic effects [8,21-25].

To the best of our knowledge there is no study on the modifying effects of several chemical compounds against CP-induced genotoxicity in exfoliated bladder cells of mice.

Maximum genotoxic response in exfoliated bladder cells of mice has been obtained at a dose of 51.6 mg/kg CP in our preliminary experiments.

CP doses as a model mutagen in mice have been ranged from 10 to 80 mg/kg in several studies [8,21-27]. Konapacka [4] has also demonstrated that CP-induced MN frequencies in exfoliated bladder cells of mice were dose-dependent, and CP doses were 20, 40 and 80 mg/kg in her studies.

Rosin has proposed that there is a temporal pattern of MN frequency induced by doses of carcinogenic agents [3]. Optimal sampling time in our study was selected the 10th day after treatment with 51.6 mg/kg of CP. Exfoliated cells were scraped from the bladder of mice at 2, 5, 7, 10, 15 and 17 days after treatment with a 51.6 mg/kg of CP. MN frequencies (‰ ± SD) were 4.0 (1.16); 8.5 (1.00); 11.9 (0.50); 17.0 (2.68); 9.3 (1.71); 4.0 (0.50) compared with the corresponding control values 1.5 (0.58); 2.3 (1.36); 4.3 (0.50); 3.0 (1.41); 1.5 (1.0); 1.5 (0.58). Thus MN frequencies increased at 2, 5, 7 days, the greatest being on the 10th day. On days 15 and 17 they decreased significantly but without returning to control values. Our results are in agreement with those of Konapacka [4] and Konapacka et al. [5] studies. These authors have indicated that the maximal level of MN cells was found 10 days after benzo(a) pyrene, CP and gamma radiation exposure.

Different doses of vitamin C (10, 30 and 60 mg/kg) were administered to determine its possible effect against CP-induced genotoxicity and dose-response association in exfoliated bladder cells of mice in this study. It has been observed that MN frequencies decreased with increasing doses of vitamin C. The 3 doses of vitamin C that we used inhibited the CP-induced genotoxicity by 41, 51 and 58% respectively. Dose-dependent inhibitory effect of vitamin C was only observed between 60 and 10 mg/kg.

Vitamin C is one of the most important antioxidant substances. It can scavenge harmful free radical metabolites/reactive oxygen species. The main mechanism underlying the protective effect is vitamin C-mediated scavenging of the mutagenic free radicals and electrophilic metabolites [28].

CP undergoes metabolic activation to produce the active mutagenic metabolite PM. In the presence of ascorbic acid, a strong reducing agent, aldophosphamide is reduced to alcophosphamide which cannot be converted into the mutagenic metabolite PM [29]. Our results may be due to the *in vivo* inhibitory effect of ascorbic acid on the genotoxicity of CP, which are in agreement with the report of Vijayalaxmi and Venu [8].

The same authors [8] also demonstrated that doses of vitamin C (10, 30 and 60 mg/kg) have inhibitory effects by 49-72% of CP-induced MN frequencies in bone marrow cells of mice. Our data proved the anticlastogenic effect of vitamin C in bladder cells of mice and the vitamin C doses were within the dose range used in humans [30]. Our results agree with the reports of chemopreventive effects of vitamin C on different tissue types, e.g. bone marrow and spleen [8].

Some studies suggest that the protective effects of vitamin C against genotoxic damage induced by gamma radiation and mutagenic chemicals may not only depend on the dose but also on the time sequence of vitamin C administration [5,24,31]. Konapacka et al. [5] demonstrated that pretreatment with vitamin C at doses ranging from 50 to 200 mg/kg before gamma irradiation caused significant decrease in MN frequencies, but surprisingly enough, at the highest dose (400 mg/kg) it produced moderately increase in MN levels in exfoliated bladder cells of mice. The authors also showed that the highest dose of vitamin C given after irradiation significantly reduced the MN frequencies.

A possible protective effect of vitamin C against doxorubicin and megestrol acetate-induced genotoxicity was observed in human lymphocytes and bone mar-

row cells [31]. Vitamin C is a water-soluble antioxidant which acts as a first defense mechanism against mutagenic metabolites formed after CP administration and provides protection against DNA damage [32].

We administered vitamin C twice, one dose 24 h prior to CP and the second dose simultaneously with the CP administration. Our results may suggest that the protective effect of vitamin C can be ascribed to its antioxidant property.

NAC has been used for many years as a mucolytic drug. It is used at 100- 600 mg/day orally in acute and chronic bronchitis and as an antidote in paracetamol poisoning and acute intoxications with acrylonitrile and paraquat. NAC has also been used for the prevention of hemorrhagic cystitis caused by CP and ifosfamide, lung fibrosis after radiotherapy, rheumatoid arthritis and in AIDS [33]. It has been observed that NAC reduces free radicals, thereby protecting cells against oxidative damage [34]. It is also the precursor of intracellular cysteine and reduces glutathione [35]. Thus, NAC is increasingly popular for its protective effect on chemical-induced carcinogenesis, e.g. colon, lung and breast studies [36].

Recent studies suggest that NAC may inhibit cytotoxic and proliferative effects in rat bladder epithelial cells *in vitro* [37] and prevent cadmium-induced renal damage and typical morphological damages in renal tubules of rats [38,39]. We, therefore, think that it may be important to examine the modifying effect of NAC against CP-induced genotoxic damage in exfoliated bladder cells of mice *in vivo*.

In this study 3 doses of NAC (200, 400 and 800 mg/kg) inhibited the CP-induced genotoxicity by 55, 51 and 70%, respectively; however, the antigenotoxic effect of NAC was not dose-dependent. NAC levels used in this study are comparable with the concentrations of 200-1000 mg/kg in humans, showing the chemopreventive effect of NAC in animals [39-42]. There is evidence that multiple mechanisms contribute to NAC antimutagenicity and anticarcinogenicity. They include several protective mechanisms such as NAC nucleophilia, antioxidant activity, its ability to act as a precursor of intracellular reduced glutathione, modulation of detoxification and DNA repair processes [6].

It is shown that CP and its metabolite acrolein produced dose-dependent depletion of hepatic glutathione in mice and these effects of CP (or acrolein) were partially blocked by cysteine [43].

Berrigan et al. [44] have demonstrated that administration of NAC (180 mg/kg, 30 min before CP and 180 mg/kg, 30 min after CP) and CP protected against the hepatic drug metabolism inactivation and urotoxicity.

In conclusion, our study indicated that vitamin C and NAC reduced the CP-induced MN frequencies in target (bladder) cells of mice by 41-71% in all cases. The modifying effects of vitamin C and NAC against CP-induced genotoxic damage may be due to their antioxidant, nucleophilic properties and to their ability to act as precursors of glutathione.

Some recent studies suggest that combinations of antioxidants may have interactive effects in modulating genotoxic effects in non-target [45,46] and target cells [5]. The present study was designated to evaluate the individual effects of vitamin C and NAC by using MN assay in exfoliated bladder cells of mice. However, further detailed studies based on the above considerations should be carried out.

Acknowledgements

This research was financially supported by the Research Fund of the Gazi University (Grant SBE-11-/98-19).

References

1. Morse MA, Stoner GD. Cancer chemoprevention, principles and prospects. Carcinogenesis 1993; 14: 1737-1746.
2. Stich HF, San RHC, Rosin MP. Adaptation of the DNA repair and micronucleus tests to human cell suspensions and exfoliated cells. Ann NY Acad Sci 1983; 407: 93-105.
3. Rosin MP. The use of the micronucleus tests on exfoliated cells to identify anti-clastogenic action in humans; A biological marker for the efficacy of chemopreventive agents. Mutat Res 1992; 267: 265-276.
4. Konopacka M. Evaluation of frequency of micronuclei in exfoliated cells from bladder of mice treated with benzo(a) pyrene, 2-acetylaminofloren and cyclophosphamide. Cell Biol Int 1994; 18: 669-672.
5. Konopacka M, Widel M, Wolny JR. Modifying effect of vitamin C, E and beta-carotene against gamma ray-induced DNA damage in mouse cells. Mutat Res1998; 417: 85-94.
6. De Flora S, Cesarone CF, Balansky RM et al. Chemopreventive properties and mechanisms of N-acetylcysteine. The experimental background. J Cell Biochem 1995; (Suppl 22): 33-41.
7. Bonorden WR, Paria MW. Antioxidant nutrients and protection from free radicals. In: Kotsonis FN, Mackey M, Hjelle J (Eds): Nutritional Toxicology, Raven Press, New York, 1994, pp 19-47.
8. Vijayalaxmi KK, Venu R. In vivo anticlastogenic effects of L-ascorbic acid in mice. Mutat Res 1999; 438: 47-51.
9. De Flora S, D'Agostini F, Izotti A, Balansky R. Prevention by N-acetylcysteine of benzo(a)pyrene clastogenicity and DNA adducts in rats. Mutat Res 1991; 250: 87-93.
10. Countryman PJ, Heddle JA. The production of micronuclei from chromosome aberrations in irradiated cultures of human lymphocytes. Mutat Res 1976; 41: 321-332.

11. Silliphant WM, Townsend FM, Ambrogi LP (Eds). Manual of histologic and special staining technics (2nd Edn). New York, Toronto, London: McGraw Hill Book Company Inc, 1960.
12. Ferguson LR. Antimutagens as cancer chemopreventive agents in the diet. Mutat Res 1994; 307: 395-410.
13. Majer BJ, Laky B, Knasmüller S, Kassie F. Use of micronucleus assay with exfoliated epithelial cells as a biomarker for monitoring individuals at elevated risk of genetic damage and in chemoprevention trials. Mutat Res 2001: 489(2-3); 147-72
14. Burgaz S, Cakmak G, Erdem O, Yilmaz M, Karakaya AE. Micronuclei frequencies in exfoliated nasal mucosa cells from pathology and anatomy laboratory workers exposed to formaldehyde. Neoplasma 2001; 48: 144-147.
15. Burgaz S, Karahalil B, Bayrak P et al. Urinary cyclophosphamide excretion and micronuclei frequencies in peripheral lymphocytes and in exfoliated buccal epithelial cells of nurses handling antineoplastics. Mutat Res 1999; 439: 97-104.
16. Burgaz S, Iscan A, Buyukbingol ZK, Bozkurt A, Karakaya AE. Evaluation of micronuclei in exfoliated urothelial cells and urinary thioether excretion of smokers. Mutat Res 1995; 335: 163-169.
17. Nersesyan AK. Possible role of the micronucleus assay in diagnostics and secondary prevention of cervix cancer: a mini review. Tsitol Genet 2007; 41: 64-66.
18. Chung FL, Xuy Ho CT, Desai D, Han C. Protection against tobacco-specific, nitrosamine-induced lung tumorigenesis by green tea and its components. In: Huang MT, Ho CT, Lee CT, Le CY (Eds): Phenolic compounds in food and their effects on health. Antioxidants and Cancer Prevention. Am Chem Society, Washington DC, 1992, pp 300-307.
19. Crook TR, Souhami RL, McLean AEM. Cytotoxicity, DNA crosslinking and single strand breaks induced by activated cyclophosphamide and acrolein in human leukemia cells. Cancer Res 1986; 46: 5029-5034.
20. Sakata T, Smith RA, Garland, Cohen SM. Rat urinary bladder epithelial lesions induced by acrolein. J Environ Pathol Toxicol Oncol 1989; 9: 159-169.
21. Salvadori DMF, Riberio LR, Oliveira MDM, Peneria CAB, Becak V. The protective effect of B-carotene on genotoxicity induced by cyclophosphamide Mutat Res 1992; 265: 237- 244.
22. De Kristina A, Agarwal K, Mukherjec A, Sengupta D. Inhibition by capsaicin against cyclophosphamide-induced clastogenicity and DNA damage in mice. Mutat Res 1995; 335: 253-258.
23. Roberts JC, Francetic DJ. Chemoprotection against cyclophosphamide-induced urotoxicity. Anticancer Res 1994; 14: 383-388.
24. Chorvatovicova D, Navarova J. Suppressing effects of glucan on micronuclei induced by cyclophosphamide in mice. Mutat Res 1992; 282: 147-150.
25. Chorvatovicova D, Bauer V. Stobadine-inhibitor of cyclophosphamide-induced micronuclei in mice. Mutagenesis 1994; 9: 241-244.
26. Sen S, Mukherjee A, Agarwall K, Sharma A. Phenethyl isothiocyanate modulates clastogenicity of mitomycin C and cyclophosphamide in vivo. Mutat Res 1996; 371: 159-164.
27. Nersesyan A, Muradyan R, Arsenyan F. The influence of three newly synthesized pyrimidine-containing compounds on micronucleus induction and tumor growth. J BUON 2007; 12: 521-527.
28. Bose S, Sinha SP. Modulation of ochratoxin-produced genotoxicity in mice by vitamin C. Food Chem Toxicol 1994; 32: 533-537.
29. Roberts JC, Francetic DJ. Chemoprotection against cyclophosphamide-induced urotoxicity. Ribose-Cysteine. Anticancer Res 1994; 14: 383-388.
30. Reynolds JEF (Ed): Martindale, The Extra Pharmacopoeia (31st Edn). The Pharmaceutical Press, London, 1996, pp 427-455.
31. Amara-Mokrane YA, Lehucher-Michel MP, Balansard G, Dumenil G, Botta A. Protective effects of alpha-hederin, chlorophyllin and ascorbic acid towards the induction of micronuclei by doxorubicin in cultured human lymphocytes. Mutagenesis 1996; 11: 161-167.
32. Siddique YH, Beg T, Afzal M, Antigenotoxic effects of ascorbic acid against megestrol acetate-induced genotoxicity in mice. Hum Exp Toxicol 2005; 24: 121-127.
33. De Vires N, De Flora S. N-acetyl-l-cysteine. J Cell Biochem 1993 (Suppl 17F): 270-277.
34. De Flora S, Izotti A, Di Agostini F, Balansky RM. Mechanisms of N-acetylcysteine in the prevention of DNA damage and cancer, with special reference to smoking-related endpoints. Carcinogenesis 2001; 22: 999-1013.
35. De Flora, Izotti A, Di Agostini F, Cesarone CF. Antioxidant activity and other mechanisms of thiols involved in chemoprevention of mutation and cancer. Am J Med 1991; 91(Suppl 3c): 122-130.
36. Boone CW, Steele VE, Kelloff GI. Screening for chemopreventive (anticarcinogenic) compounds in rodents. Mutat Res 1992; 267: 251-255.
37. Wei M, Arnold L, Cano M et al. Effects of co-administration of antioxidants and arsenicals on the rat urinary bladder epithelium. Toxicol Sci 2004; 83: 237-245.
38. Chen N, Aleksa K, Woodland C, Rieder M, Koren G. N-acetylcysteine prevents ifosfamide-induced nephrotoxicity in rats. Br J Pharmacol 2008; 153: 1364-1372
39. Kaplan M, Atakan IH, Aydogdu N et al. Influence of N-acetylcysteine on renal toxicity of cadmium in rats. Pediatr Nephrol 2008; 23: 233-241.
40. Izotti A, Orlando M, Gasparini L et al. In vitro inhibition by N-acetylcysteine of oxidative DNA modifications detected by 32 P postlabelling. Free Radic Res 1998; 28: 165-178.
41. Zhao C, Shichi H. Prevention of acetaminophen-induced cataract by a combination of diallyl disulfide and N-acetylcysteine. J Ocul Pharmacol Ther 1998; 14: 345-355.
42. Balansky RM, De Flora S. Chemoprevention by N-acetylcysteine of urethane-induced clastogenicity and lung tumors in mice. Int J Cancer 1998; 77: 302-305.
43. Gurtoo HL, Hipkens JH, Sharma SD. Role of glutathione in the metabolism-depended toxicity and chemotherapy of cyclophosphamide. Cancer Res 1981; 41: 3584-3591.
44. Berrigan MJ, Marinello AJ, Pavelic Z, Williams CJ, Struck RF, Gurto HL. Protective role of thiols in cyclophosphamide-induced urotoxicity and depression of hepatic drug metabolism. Cancer Res 1982; 42: 3688-3695.
45. Di Agostini F, Balansky RM, Camoirano A, De Flora S. Interactions between N-acetylcysteine and ascorbic acid in modulating mutagenesis and carcinogenesis. Int J Cancer 2000; 88: 702-707.
46. Balansky RM, De Flora S. Interactions between N-acetylcysteine and sodium selenite in modulating the clastogenicity of urethane and 2-acetylaminofluorene in mice. Int J Cancer 2004; 108: 158-161.