IJMS
Vol 34, No 1, March 2009

Original Article

Effect of Lycopene on Cyclophosphamide-Induced Hemorrhagic Cystitis in Rats

Akram Jamshidzadeh[1],
Hossein Niknahad[1], Negar Azarpira[2],
Afshin Mohammadi- Bardbori[1],
Maryam Delnavaz[1]

Abstract

Background: Cylophosphamide is used alone or in combination with other drugs for treatment of neoplastic diseases. Hemorrhagic cystitis is a major potential toxicity and dose limiting side effect of cyclophosphamide. The aim of this study was to evaluate the effects of lycopene compared with some antioxidants for the prevention of cyclophosphamide induced hemorrhagic cystitis in rats.

Methods: In this study, male Sparague-Dawley rats divided into 17 groups of six animals. Group 1 received saline (10 ml/kg, i.p) as normal control**,** group 2 received cyclophosphamide (200 mg/kg, i.p) as a single dose, groups 3-10 received Mesna (40 mg/kg, i.p), N-acetylcysteine (100 mg/kg i.p), dithiotheritol (50 mg/kg, i.p), L-carnitine (200 and 400 mg/kg, i.p), grape seed extract (500 mg/kg i.p) and lycopene (0.1 and 0.5 mg/kg, i.p) alone. Groups 11-17 received Mesna (40 mg/kg, i.p), N-acetylcysteine (100 mg/kg, i.p), dithiotheritol (50 mg/kg, i.p), L-carnitine (400 mg/kg, i.p), grape seed extract (500 mg/kg, i.p) and lycopene (0.1 and 0.5 mg/kg, i.p), 5 minutes before, and 2 and 6 hours after administration of 200 mg/kg cyclophosphamide. Pathological and biochemical analysis was evaluated 24 hours after cyclophosphamide administration.

Results: Mesna and N-acetylcysteine resulted in some but not full protection against cyclophosphamide toxicity compared to the controls. Lycopene (0.1 and 0.5 mg/kg) was efficient in protecting the bladder from cyclophosphamide induced hemorrhagic cystitis. However, dithiotheritol, L- carnitine and grape seed extract did not prevent hemorrhagic cystitis.

Conclusion: Our results suggest that pre and co- treatment of lycopene (0.1 and 0.5 mg/kg) with cyclophosphamide may have therapeutic potential to inhibit the hemorrhagic cystitis by cyclophosphamide.

Iran J Med Sci 2009; 34(1): 46-52.

Keywords ● Cyclophosphamide ● cystitis ● hemorrhagic ● lycopene ● rat ● antioxidant

[1]Department of Pharmacology and Toxicology,
School of Pharmacy,
[2]Transplant Research Center,
Nemazi Hospital,
Shiraz University of Medical Sciences,
Shiraz, Iran.

Correspondence:
Akram Jamshidzadeh PhD,
Department of Pharmacology and Toxicology,
Faculty of Pharmacy,
Shiraz University of Medical Sciences,
Shiraz, Iran.
Tel: +98 711 2424126
Fax: +98 711 2426070
Email: ajamshid@sums.ac.ir
Submitted: 18 June 2008
Revised: 2 November 2008
Accepted: 21 December 2008

Introduction

Cyclophosphamide (CP) is an alkylating anticancer drug widely used in the treatment of chronic and acute leukemia, multiple myeloma, and lymphomas.[1] CP is also an immunosuppressant and immunomodulator,[2]

which results in growth retardation and failure of neurulation. The urological side effects of CP are major limiting factors for its use. These side effects include transient irritative voiding symptoms including dypsia, hemorrhagic cystitis, bladder fibrosis, necrosis, contracture and vesicometral flux.[3]

CP also induces a wide toxicity to normal cells in human and experimental animals. The mechanisms whereby CP causes toxicity are poorly understood; however, numerous studies have shown that CP exposure enhances production of intracellular reactive oxygen species (ROS), suggesting that biochemical and physiological disturbances may result from oxidative stress.[4-6] CP exerts cytotoxicity by changing the activities of enzymes and levels of non-enzymic antioxidants.[7-11] Recent studies have indicated that nitric oxide (NO) may play an important role in the pathogenesis of the cystitis associated with cyclophosphamide treatment.[12] Production of NO through increased expression of inducible nitric oxide synthase (iNOS) is an important factor in cyclophosphamide-induced inflammatory changes in rat bladder.[13]

Acrolein is a urinary metabolite of cyclophosphamide that has been reported to be the causative agent of hemorrhagic cystitis. A direct cytotoxic effect of acrolein, however, has not yet been demonstrated.[14]

The role of tissue antioxidants becomes important in the prevention of such damage. Recently, there has been increasing interest in searching for potential drugs of plant origin capable of minimizing the toxicity induced by chemotherapy to normal cells without compromising its anti-neoplastic activity.

Lycopene, the most prominent carotenoids in tomato, has attracted considerable interest for its biological and physicochemical properties.[15] Lycopene exhibits the highest overall single oxygen-quenching carotenoids.[16]

Some studies have shown that consumption of tomatoes and processed tomato products that abundantly contain lycopene produce increased lycopene blood levels that may be associated with reduced oxidative damage to lipids, proteins and DNA.[17] In recent years, a great interest has emerged concerning the protective biochemical function of the natural antioxidants contained in dietary plants that are candidates for prevention or protection of oxidative damage caused by free radical species.[18,19]

In the present study, the effect of lycopene was compared with other antioxidants on hemorrhagic cystitis induced by cyclophosphamide in rats.

Materials and Methods

Chemicals

Grape seeds (*Vitis Vinifera*) were collected from Sardasht (north-west Iran) in late September and it was authenticated by the Botany department, Shiraz University, Iran. CP and 2-mercaptoethanesulfonic acid (Mesna) were purchased from Baxter (Germany), lycopene from Nature‘s life larkspur (U.S.A) and other chemicals were obtained from Merck (Germany).

Animals

The male Sprague-Dawley rats (200-250 g) were obtained from the Laboratory Animals Research Center of Shiraz University of Medical Sciences. The rats were housed in environmentally (25 °C) and air humidity controlled room (60%) and kept on standard laboratory diet and were maintained on a 12–hour light-dark cycle for one week prior to the experiments. The animals received standard laboratory chaw and tap water *ad libitum*.

Experimental design

In the present study, the rats were randomly divided into 17 groups of 6 animals. Group 1 received normal saline [10 ml/kg, intraperitoneally (i.p)], group 2 received CP (200 mg/kg, i.p),[20] as a single dose, groups 3-10 received mesna (40 mg/kg, i.p), N-acetylcysteine (NAC) (100 mg/kg, i.p), dithiothreitol (DTT) (50 mg/kg, i.p), L-carnitine (200 and 400 mg/kg, i.p), grape seed extract (GSE; 500 mg/kg, i.p), and lycopene (0.1 and 0.5 mg/kg, i.p). Groups 11-17 received mesna (40 mg/kg, i.p), NAC (100 mg/kg, i.p), DTT (50 mg/kg, i.p), L-carnitine (400 mg/kg, i.p), GSE (500 mg/kg, i.p), and lycopene (0.1 and 0.5 mg/kg, i.p), 5 minutes prior to and 2 and 6 hours after administration of 200 mg/kg CP. All doses of antioxidants used in the present study were selected based upon previous studies. Twenty four hours after administration of CP (200 mg/kg), the animals were anesthetized (thiopental, 50mg/kg body wt, i.p) and euthanized, and the bladders were collected for biochemical (total tissue levels of glutathione and malondialdehyde) and histopathological examinations.

Preparation of extract

The seeds of *Vitis Vinifera* (Nigra) used in the present study were washed thoroughly under tap water, shade-dried and powdered. The powder (250 g) was successively extracted with 1000 ml methanol (80%) overnight with constant stirring. The filtrate was then concentrated and the solvent was evaporated under

reduced pressure in a rotary evaporator. The extract yield was 10% (w/v).

Histopathological Studies

Upon removing, the bladders were fixed in 10% formalin for at least 24 hours. The paraffin sections were prepared (automatic tissue processor) and cut into 5 µm sections using a rotary microtome. The sections were stained with hematoxylin-eosin and studied for histopathological changes. Both macroscopic (edema and hemorrhage) and microscopic histological changes were evaluated according to the Gray et al.[20] criteria as follows: Edema was considered sever (3+) when fluid was seen externally and internally on the wall of the bladder, moderate edema (2+) when confined to the internal mucosa, mild edema (1+) that scored between (2+) and (0) and absent (0) when no edema was observed. Hemorrhage was scored as follows: (3+) with intravesical clots, (2+) with mucosal hematomas, (1+) with telangiectasia or dilatation of the bladder vessels, and (0) or normal. Histopathology was scored as follows: (0)=normal epithelium and absence of inflammatory cell infiltration and ulceration, (1+)=mild changes involving reduction of epithelial cells, flattening with submucosal edema, mild hemorrhage and few ulcerations, (2+)=severe changes including mucosal erosions, inflammatory cell infiltration, fibrin deposition, hemorrhage, and multiple ulcerations. The semiquantitative macroscopic and microscopic observations are reported as medians and range.

Determination of Lipid Peroxidation

Lipid peroxidation was determined by quantifying malondialdehyde (MDA) concentrations spectrophotometrically by measuring the optical density of the red-colored product with thiobarbituric acid (TBA). Briefly, a portion of the bladder tissue was combined with 4.5 ml of 0.25 M sucrose, minced and gently homogenized, and centrifuged at 2000 rpm for 30 min. One tenth of the supernatant was treated with a buffer containing 0.75 ml of TBA (8.1% (W/V), 0.75 ml of acetic acid 20% (pH 3.5) and 0.1 ml of sodium dodecylsulfate. The volume of this solution was brought to 2 ml with water and the mixture was heated in a boiling water bath for 60 minutes. The optical density was measured at 532 nm using a Beckman DU®-7 spectrophotometer.[21]

Glutathione Sulfur Hydrogen or Reduced Glutathione (GSH) Determination

Reduced glutathione was determined using the glutathione reductase 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) recycling procedure.[22] Briefly, the tissue was homogenized with a buffer containing EDTA (0.2M) to obtain 4:100 (W/V) whole homogenate. Then 1.5 ml of the suspension was combined with a buffer containing 2.5 ml of distilled water, 0.5 ml of Trichloroacetic acid (50%), and centrifuged at 3000 rpm for 15 min. One ml of the supernatant was mixed with Tris buffer solution (0.4 M, pH 8.9) and 0.1 ml of DTNB (0.01 M). The optical density was measured after 5 min at 412 nm using a Beckman DU®- 7 spectrophotometer.[23]

Statistical Analysis

All values are expressed as mean± standard error (SEM) of the six samples. Analysis of variance (ANOVA) followed by Student Newmans-Keuls test was used to evaluate the significance of the results obtained. All the computations were performed using SPSS software version 14. Histological changes were evaluated according to the criteria of Gray et al.[20]

Results

Histopathology

Cystitis observed 24 hours after CP administration was characterized macroscopically by the presence of severe edema, receiving a score of (3+), and by extensive hemorrhage with mucosal hematomas, receiving a score of (3+). The cystitis was significantly ($P < 0.05$) different from the control group that received a score of (0) for edema and hemorrhage. Macroscopic analysis of the bladder 24 hours after CP injection revealed that pre-treatments with mesna, NAC, and lycopene (0.5 mg/kg) significantly reduced the analyzed parameters (table 1). According to Gray's histopathological criteria, compared with normal bladders, microscopic analysis of the bladders of rats injected 24 hours before with 200 mg/kg of CP revealed extensive cystitis characterized by acute inflammation with vascular congestion, edema, hemorrhage with fibrin deposition, neutrophil infiltration, and epithelial denudation, which equals a score of (2+). These alterations were almost abolished by the treatment of the CP-injected rats with mesna, NAC, and lycopene (0.5 mg/kg) (1+). No significant differences were found between lycopene (0.1 mg/kg) with CP (table 1). No significant differences were found between all the compounds used except CP, when compared with controls.

Glutathione Content

Table 2 shows that the bladder glutathione GSH content was significantly decreased in CP group, compared with the control group. Grape seed, DTT and L-carnitine failed to prevent the

Table 1: Histopathological evaluation of the effects of lycopene and some antioxidants on cyclophosphamide-induced hemorrhagic cystitis in rats.

Groups	Macroscopic analysis		Microscopic analysis
	Edema	Hemorrhage	
Control	0	0	0
CP (200 mg/kg)	3+	3+	3+
CP+ Mesna (40 mg/kg)	1+	1+	1+
CP+NAC (100 mg/kg)	1+	1+	1+
CP+DTT (50 mg/kg)	3+	3+	2+
CP+ Carnitine (500 mg/kg)	3+	3+	2+
CP+ GSE (500 mg/kg)	3+	3+	2+
CP+ lycopene (0.1 mg/kg)	2+	2+	1+
CP+ lycopene (0.5 mg/kg)	1+	1+	1+

0: absent, 1+: mild, 2+: moderate, 3+: severe, CP: Cyclophosphamide, NAC: N-acetylcysteine, DTT: Dithiothreitol, GSE: Grape seed extract

CP–induced decreases of GSH but lycopene, NAC, and mesna ameliorated CP-induced decreases of GSH ($P<0.05$). No significant changes were found in GSH content between all the compounds used except CP when compared with the controls ($P<0.05$).

Lipid Peroxidation Content

Table 2 shows that the bladder lipid peroxidation was significantly increased in CP group when compared with the controls. Grape seed, DTT and L-carnitine failed to prevent the CP–induced increases of MDA concentration. However, lycopene, NAC and mesna ameliorated CP-induced increases of MDA concentration ($P<0.05$). No significant changes were found in MDA level between all the compounds used except CP, when compared with the controls ($P<0.05$).

Discussion

Development of a non-toxic selective cytoprotective agent that preferentially protects normal tissues from chemotherapy-induced toxicity, without protecting malignant tissues, represents a major challenge in cancer chemotherapy research.

CP undergoes metabolic activation catalyzed by liver cytochrome P450 enzymes to form 4-hydroxycyclophosphamide that produces a reactive alkylating agent phosphoramide mustard via beta eliminates Acrolein.[24] It has been suggested that acrolein is responsible for urinary bladder toxicity of CP.[25] High NO level has been found in CP treated rats.[26] The reaction of NO with superoxide results in the generation of peroxynitrite that may mediate the pathogenesis of CP-induced cystitis.[12]

Cystitis observed 24 hours after CP administration was characterized macroscopically by the presence of severe edema, hemorrhage with mucosal hematomas, and intravesical clots, receiving a score of 3. This score was found different from the control group that received a score of zero for edema and hemorrhage. Treatment with mesna and NAC reduced the intensity of cystitis (table 1) that is consistent with other studies. Our findings indicate that lycopene could be a useful compound, compared with dithiothreitol, L- carnitine, and grape seed extract, in the preventive management of CP-induced hemorrhagic cystitis.

Malondialdehyde is commonly known as a marker of oxidative stress and antioxidant status in cells. CP caused a significant increase in MDA level in the bladder. The oxidative products of CP responsible for elevation of MDA and generation of ROS result in inflammation, thus disturbing the overall redox cycling of the bladder.[27] Increase in MDA concentration reported here might be the result of

Table 2: Protective effect of lycopene and some antioxidants against cyclophosphamide- induced hemorrhagic cystitis in rats.

Groups	Biochemical Analysis Mean± SEM	
	MDA (ng / mg tissue)	GSH (µg/mg tissue)
Control	0.063±0.001	4.293±0.19
CP (200 mg/kg)	0.228±0.001***	0.496±0.280***
CP+ Mesna (40 mg/kg)	0.067±0.003††	3.271±0.170††
CP+ NAC (100 mg/kg)	0.070±0.003††	1.989±0.963†
CP+DTT (50 mg/kg)	0.195±0.001	0.438±0.352
CP+ Carnitine (500 mg/kg)	0.130±0.001†	0.499±0.000
CP+ GSE (500 mg/kg)	0.130±0.001†	0.438±0.000
CP+ lycopene (0.1 mg/kg)	0.065±0.001††	3.554±0.000††
CP+ lycopene (0.5 mg/kg)	0.068±0.001††	3.654±0.033††

*** ($P<0.001$) significantly different when compared with controls. † ($P<0.05$), †† ($P<0.01$) significantly different when compared with CP alone.

increased production of free radicals and/or decrease in antioxidant status (table 2).

GSH is an important biomarker of cellular antioxidative status. In the urinary bladder, depleted GSH levels resulting from CP treatment has been reported,[28] which is consistent with our results. Depletion of GSH content may be attributed to the direct conjugation of CP with free or protein bound SH groups (table 2). A number of GSH-elevating compounds have been found to be effective in reducing CP toxicity in animals. Mesna (2-mercaptoethanesulfonic acid) that is a thiol containing compound was studied in clinical trials as a systemic uroprotective agent in the late 1970's and shortly became the drug of choice for this purpose.[29] However, mesna is not effective when the lesions have been established. Additionally, mesna decreases the incidence of cystitis by about 5%. These findings indicate several shortcomings for using mesna and favor search for newer uroprotective agents, especially those with a potential to augment GSH.[30]

NAC has been used clinically as an antioxidant in conditions characterized by GSH depletion and is the drug of choice as an antidote for acetaminophen poisoning both in adults and children.[31] NAC enhances the biosynthesis of GSH by donating the cysteine that is a precursor.[32]

Depletion of thiols along with free radical generation has been implicated in cyclophosphamide-induced urotoxicity. S-Allylcysteine, an organosulfur compound of aged garlic extract has shown to regulate the thiol status of the cell and scavenge free radicals.[33]

The protection offered by mesna and NAC against CP-induced bladder damage in the present study was in accordance with previous studies.

A number of herbal extracts and their isolated constituents have also shown protective effect against CP-induced urotoxicity.[34,35] Quercetin has shown protective effect against CP-induced hemorrhagic cystitis.[36] Antioxidants such as α-tocopherol, β-carotene, epigallocatechin, quercetin and melatonin, have been shown to protect against bladder damage when combined with mesna.[37]

Recent studies have indicated the potential health benefits of tomato enriched diets and tomato products. Tomato is also the main dietary source of lycopene, the most prominent carotenoid in tomatoes and the most potent in vitro antioxidant among carotenoids.[16] Lycopene has been shown a protective effect against toxicity induced by other anti-tumor drug.[38-40] Lycopene is one of the most potent antioxidants among the dietary carotenoids due to its multiple conjugated double bounds. Lycopene also has the strongest singlet oxygen-quenching ability compared with other carotenoids.[41] Besides quenching singlet molecular oxygen and peroxyl radicals,[42] strong interaction of lycopene has been shown to occur with other active oxygen species such as H_2O_2,[43] that generates hydroxyl radicals known to induce membrane lipid peroxidation and DNA strand scission.[44] Our results suggest that lycopene administration may have therapeutic effect to inhibit the cyclophosphamide side effect (tables 1 and 2).

Grape seed extract contains proanthocyanidins as well as other natural phenolic compounds and exhibits antioxidant properties. The antioxidant activity of grape seed extract makes it a candidate as an additive to foods and beverages to retard deterioration. It is possible that the antioxidant activity of grape seed extract ingested with these foods would also support physiologic defenses against in vivo generated free radical species. However, grape seed extract contain minor amounts (0.1–1.0%) of quercetin and its glycosides are substances with suspected mutagenic activity.[45] Our results suggest that grape seed extract, DTT and L-carnitine administration are unable to inhibit cyclophosphamide-induced hemorrhagic cystitis. The fact that GSE exhibits antioxidant effect in vitro but not in vivo suggests that these uroprotectant intracellular concentrations are not great enough to neutralize toxic metabolites within renal tubule cells (tables 1 and 2).

We suggest that lycopene or tomato extract supplement might offer a simple inexpensive method that can be used with cyclophosphamide therapy to decrease its side effects.

Conflict of Interest: None declared

References

1 Zaki EL, Springate JE, Taub M. Comparative toxicity of ifosfamide metabolites and protective effect of mesna and amifostine in cultured renal tubule cells. *Toxicol In Vitro* 2003; 17: 397-402.

2 Latorre AO, Hueza IM, Górniak SL. Association of Ipomoea carnea and BCG reduces birth defects caused by cyclophosphamide in rats. *Life Sci* 2007; 80: 430-5.

3 Levine LA, Richie JP. Urological complications of cyclophosphamide. *J Urol* 1989; 141: 1063-9.

4 Das UB, Mallick M, Debnath JM, Ghosh D. Protective effect of ascorbic acid on cyclophosphamide-induced testicular gametogenic

and androgenic disorders in male rats. *Asian J Androl* 2002; 4: 201-7.
5 Ghosh D, Das UB, Ghosh S, et al. Testicular gametogenic and steroidogenic activities in cyclophosphamide treated rat: a correlative study with testicular oxidative stress. *Drug Chem Toxicol* 2002; 25: 281-92.
6 Manda K, Bhatia AL. Prophylactic action of melatonin against cyclophosphamide-induced oxidative stress in mice. *Cell Biol Toxicol* 2003; 19: 367-72.
7 Selvakumar E, Prahalathan C, Sudharsan PT, Varalakshmi P. Chemoprotective effect of lipoic acid against cyclophosphamide-induced changes in the rat sperm. *Toxicology* 2006; 217: 71-8.
8 Selvakumar E, Prahalathan C, Varalakshmi P. Modification of cyclophosphamide-induced clastogenesis and apoptosis in rats by α-lipoic acid. *Mutat Res-Genet Toxicol Environ* 2006; 606: 85-91.
9 Revnic C, Revnic F, Botea S. Evaluation of antioxidant potential of vitamin E and mRNA synthesis in Cyclophosphamide treated rat brain. *J Neurol Sci* 2005; 238: S525-6.
10 Lopez S, Luderer U. Effects of cyclophosphamide and buthionine sulfoximine on ovarian glutathione and apoptosis. *Free Radic Biol Med* 2004; 36: 1366-77.
11 Medina S, Martinez M, Hernanz A. Antioxidants inhibit the human cortical neuron apoptosis induced by hydrogen peroxide, tumor necrosis factor alpha, dopamine and beta-amyloid peptide 1-42. *Free Radic Res* 2002; 36: 1179-84.
12 Alfieri A, Cubeddu LX. Role of NK1 receptors and nitric oxide on cyclophosphamide-induced bladder toxicity. *J Pharmacol Exp Ther* 2000; 295: 824-8.
13 Xinyun Xu, Luigi X, Cubeddu AM. Expression of inducible nitric oxide synthase in primary culture of rat bladder smooth muscle cells by plasma from cyclophosphamide-treated rats. *Eur J Pharmacol* 2001; 416: 1-9.
14 Dechant KL, Brogden RN, Pilkington T, Faulds D. Ifosfamide/mesna. A review of its antineoplastic activity, pharmacokinetic properties and therapeutic efficacy in cancer. *Drugs* 1991; 42: 428-67.
15 Djuric Z, Powell LC. Antioxidant capacity of lycopene-containing foods. *Int J Food Sci Nutr* 2001; 52: 143-9.
16 Di Mascio P, Kaiser S, Sies H. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch Biochem Biophys* 1989; 274: 532-8.
17 Karas M, Amir H, Fishman D, et al. Lycopene interferes with cell cycle progression and insulin-like growth factor I signaling in mammary cancer cells. *Nutr Cancer* 2000; 36:101-11.
18 Roa AV, Agarwal S. Role of antioxidant lycopene in cancer and heart diseas. *J Am Coll Nutr* 2000; 19: 563-9.
19 Toniolo P, Van Kappel AL, Akhmedkhanov A, et al. Serum carotenoids and breast cancer. *Am J Epidemiol* 2001; 153: 1142-7.
20 Gray KJ, Engelmann UH, Johnson EH, Fishman IJ. Evaluation of misoprostol cytoprotection of the bladder with cyclophosphamide (Cytoxan) therapy. *J Urol* 1986; 136:497-500.
21 Jamall IS, Smith JC. Effects of cadmium on glutathione peroxidase, superoxide dismutase, and lipid peroxidation in the rat heart: a possible mechanism of cadmium cardiotoxicity. *Toxicol Appl Pharmacol* 1985; 80: 33-42.
22 Tietze F. Enzymatic method for quantitative determination of nanogram amounts of total and oxidized glutathione. *Anal Biochem* 1969; 27: 502-22.
23 Sedlak J, Lindsay RH. Estimation of protein bound and nonprotein sulfhydryl groups in tissues using Ellman's reagent. *Anal Biochem* 1968; 25: 192-205.
24 Kehrer JP, Biswal SS. The molecular effects of acrolein. *Toxicol Sci* 2000; 57: 6-15.
25 Nicol D. Cyclophosphamide and the urinary tract. *Intern Med J* 2002; 32: 199-201.
26 Matar P, Rozados VR, González AD, et al. Mechanism of antimetastatic immunopotentiation by low-dose cyclophosphamide. *Eur J Cancer* 2000; 36:1060-6.
27 Cooper JA, Merrill WW, Reynolds HY. Cyclophosphamide modulation of bronchoalveolar cellular populations and macrophage oxidative metabolism: possible mechanisms of pulmonary pharmacotoxicity. *Am Rv Respir Dis* 1986; 134: 108-14.
28 Manesh C, Kuttan G. Effect of naturally occurring isothiocyanates in the inhibition of cyclophosphamide-induced urotoxicity. *Phytomedicine* 2005; 12: 487-93.
29 Katz A, Epelman S, Anelli A, et al. A prospective randomized evaluation of three schedules of mesna administration in patients receiving an ifosfamide-containing chemotherapy regimen: sustained efficiency and simplified administration. *J Cancer Res Clin Oncol* 1995; 121: 128-31.
30 Elias AD, Eder JP, Shea T, et al. High dose ifosfamide with mesna uroprotection: a phase I study. *J Clin Oncol* 1990; 8: 170-8.
31 Chen N, Aleksa K, Woodland C, et al. N-Acetylcysteine prevents ifosfamide-induced

nephrotoxicity in rats. *Br J Pharmacol* 2008; 153: 1364-72.
32 De Vries N, De Flora S. N-acetyl-l-cysteine. *J Cell Biochem Suppl* 1993; 17F: 270-7.
33 Bhatia K, Ahmad F, Rashid H, Raisuddin S. Protective effect of S-allylcysteine against cyclophosphamide-induced bladder hemorrhagic cystitis in mice. *Food Chem Toxicol* 2008; 46: 3368-74.
34 Davis L, Kuttan G. Effect of Withania somnifera on cyclophosphamide-induced urotoxicity. *Cancer Lett* 2000; 148: 9-17.
35 Vieira MM, Macedo FY, Filho JN, et al. Ternatin, a flavonoid, prevents cyclophosphamide and ifosfamide-induced hemorrhagic cystitis in rats. *Phytother Res* 2004; 18: 135-41.
36 Ozcan A, Korkmaz A, Oter S, Coskun O. Contribution of flavonoid antioxidants to the preventive effect of mesna in cyclophosphamide-induced cystitis in rats. *Arch Toxicol* 2005; 79: 461-5.
37 Sadir S, Deveci S, Korkmaz A, Oter S. Alpha-tocopherol, beta-carotene and melatonin administration protects cyclophosphamide-induced oxidative damage to bladder tissue in rats. *Cell Biochem Funct* 2007; 25: 521-6.
38 Yilmaz S, Atessahin A, Sahna E, Karahan I, Ozer S. Protective effect of lycopene on adriamycin-induced cardiotoxicity and nephrotoxicity. *Toxicology* 2006; 218: 164-71.
39 Ferreira AL, Salvadori DM, Nascimento MC, et al. Tomato-oleoresin supplement prevents doxorubicin-induced cardiac myocyte oxidative DNA damage in rats. *Mutat Res* 2007; 631: 26-35.
40 Jamshidzadeh A, Baghban M, Azarpira N, et al. Effects of tomato extract on oxidative stress induced toxicity in different organs of rats. *Food Chem Toxicol* 2008; 46: 3612-5.
41 Stahl W, Sies H. Physical quenching of singlet oxygen and cis-trans isomerization of carotenoids. *Ann N Y Acad Sci* 1993; 691: 10-9.
42 Gerster H. The potential role of lycopene for human health (review). *J Am Coll Nutr* 1997; 16: 109-26.
43 Agarwal S, Rao AV. Tomato lycopene and its role in human health and chronic diseases. *CMAJ* 2000; 163: 739-44.
44 Lu Y, Etoh H, Watanabe N, et al. Anew carotenoids, hydrogen peroxide oxidation products from lycopene. *Biosci Biotech Biochem* 1995; 59: 2135-55.
45 Erexson GL. Lack of in vivo clastogenic activity of grape seed and grape skin extracts in a mouse micronucleus assay. *Food Chem Toxicol* 2003; 41: 347-50.