Modulation of the tissue defense system by squalene in cyclophosphamide induced toxicity in rats

Subramanian Senthilkumar[1], Kesavarao Kumar Ebenezar[1, 2], Venkatachalem Sathish[1, 3], Surinderkumar Yogeeta[1], Thiruvengadam Devaki[1]

[1]Department of Biochemistry, University of Madras, Guintry campus, Chennai, India
[2]Department of Physiology and Biophysics, University of Calgary, Canada
[3]Department of Physiology and Pharmacology, University of Western Ontario, London, Canada

Corresponding author:
Thiruvengadam Devaki, PhD
Department of Biochemistry
University of Madras
Guindy campus
Chennai – 600025
Tamil Nadu, India
Phone: +91 44 223 512 69 x 231
Fax: +91 44 223 524 94
E-mail: devakit@yahoo.co.uk

Abstract

Introduction: Antineoplastic drug, Cyclophosphamide (CP), is a widely used drug that causes toxicity through its metabolites, phosphoramide mustard and acrolein. Squalene (SQ), an intermediate in the cholesterol metabolism has antioxidant and membrane stabilizing property. In the present study, the protective role of SQ towards the tissue defense system of the liver and kidney in the toxicity induced by CP was assessed.
Material and methods: Normal Wistar albino rats were administered CP in a dose of 150 mg/kg b.wt., i.p., twice, for 2 consecutive days to induce toxicity. SQ, in a dose of 0.4 ml/day/rat p.o. was used to treat the toxicity induced by CP.
Results: Significantly decreased activities of enzymic antioxidants [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-s-transferase (GST) and glutathione reductase (GR)], decreased levels of reduced glutathione and increased levels of thiobarbutric acid reactive substance (TBARS) were observed. These pathological alterations were significantly normalized during the treatment of SQ.
Conclusions: CP toxicity increased the free radical levels in the tissues and affected the activities of the enzymic antioxidants. Increased levels of TBARS [a measure of lipid peroxidation (LPO)] and decreased levels of GSH (due to utilization for detoxification process) evidenced the damage to these tissues. Protection exerted by SQ could be due to free radical quenching, providing additional alkylation site to CP metabolites and by inducing enzymic antioxidant production in these tissues. In conclusions improved antioxidant defense system in the liver and kidney of the experimental rats confirms the protective role of SQ against CP induced toxicities.

Key words: enzymic antioxidants, TBARS, reactive oxygen species, free radicals, liver, kidney.

Introduction

A free radical is any atom or molecule, capable of independent existence that has one or more unpaired electrons. If uncontrolled by the protective mechanisms, it leads to a pathological effect which causes damage to cellular membranes, proteins and nucleic acids [1]. The major limitation of cancer chemotherapy is the injury of the normal tissue, leading to multiple organ toxicity [2, 3]. CP is an alkylating agent, the most commonly used anticancer and immunosuppressant drug. It is used for the treatment of chronic and

acute leukemias, multiple myeloma, lymphomas, and rheumatic arthritis and also in the preparation for bone marrow transplantation [4, 5]. High-dose chemotherapy administered in bone marrow transplantation regimens commonly induces nausea, emesis, oropharyngeal inflammation, abdominal pain and diarrhea [6]. Phosphoramide mustard and acrolein are the two active metabolites of CP formed by hepatic microsomal cytochrome P_{450} mixed functional oxidase system [7]. CP's antineoplastic effects are associated with the phosphoramide mustard, while the acrolein is linked with its toxic side effects [8]. Chemotherapy and radiation therapy are associated with increased formation of reactive oxygen species (ROS) and depletion of critical plasma and tissue antioxidants [9]. Two reactive moieties of acrolein, an aldehyde group and an unsaturated bond, have the potential to cross-link to nucleophilic groups on two different proteins [10]. Acrolein is a relatively long-lived molecule that might diffuse some distance before reacting with proteins [11]. Acrolein interferes with the tissue antioxidant defense system [12], produces highly reactive oxygen free radicals [13] and is mutagenic to mammalian cells [14]. Due to the highly reactive nature, free radicals can readily combine with other molecules, such as enzymes, receptors, and ion pumps, causing oxidation directly, and inactivating or inhibiting their normal functions [15].

Liver disorders were observed in the elevated therapeutic dose of CP [16-19]. The nephrotoxicity of CP was evidenced by the proximal tubular damage, a significant reversible depolarization and a decrease in conductance [20, 21]. CP induced urinary bladder inflammation has demonstrated alterations in neurochemical [22] electrophysiological properties [23] of the bladder.

Effects due to CP toxic metabolites could be avoided by detoxifying with agents which are able to conjugate or quench these toxic metabolites. An antioxidant agent like amifostine has a cytoprotective action against platinum-induced renal toxicity, was avoided due to its toxicities like hypocalcaemia, anxiety and hypotension [24, 25]. Other cytoprotective agents such as sodium thiosulphate, mesna and procainamide are not approved for wide clinical use due to lack of efficacy and non-selective cytoprotection against toxicity induced by platinum and alkylating agents in tumour tissues [26]. In a recent work, it has been concluded that hydralazine prevents the protein cross-linking against acrolein mediated toxicity [27]. Therefore, there is a need for a novel agent, which would protect the normal tissue from chemotherapy-induced toxicity without tumour protection and tumour growth stimulation properties.

SQ, the intermediate of the cholesterol metabolism, is an isoprenoid compound having six isoprene units. SQ has been reported to possess antioxidant and membrane stabilizing properties [28-30]. In vitro experimental evidence indicates that the SQ is a highly effective singlet oxygen scavenging agent [31]. The protective activity of SQ against radiation-induced injury was demonstrated in a mouse model [32]. Several experimental models demonstrated the detoxifying activities of SQ against a wide range of chemicals and a sink for highly lipophilic xenobiotics, assisting in their elimination from the body [33-35]. In our previous studies, it has been proved that the toxicity induced within 10 days by the administration of CP was attenuated by the treatment of SQ [36, 37]. The present study was designed to evaluate the protective efficacy of SQ towards the antioxidant defensive mechanism in CP induced toxicity in the liver and kidney of the experimental rats.
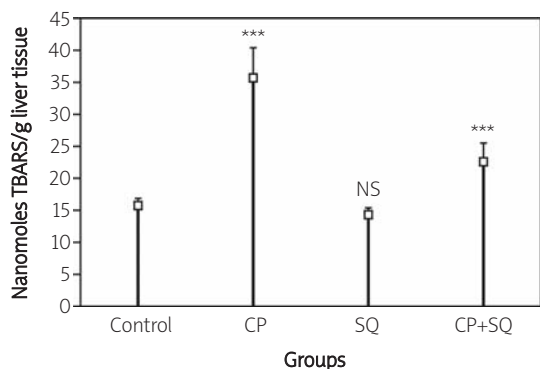
Materials and methods

Chemicals and drugs

Cyclophosphamide (Ledoxan®) was purchased from Dabur Pharma Limited, New Delhi, India. Squalene (≥97% by GC) was procured from Sigma Chemicals Company, St. Louis, MO, USA. All other chemicals and solvents used were of the highest purity and analytical grade.

Experimental design

Male albino Wistar rats (150±10 g) procured from Tamilnadu University for Veterinary and Animal Sciences, Madhavaram, Chennai, India were used for the study. Animals were fed with commercially available standard rat pelleted feed. The feed and water were provided *ad libitum*. The rats were housed under conditions of controlled temperature (25±2°C) and were acclimatized to 12:12 h light: dark cycles. Animal experiments were conducted according to the guidelines of the Institutional Animal Ethics Committee (Approval No. 01/006/06).

The rats were divided into four groups of six animals each. Group I served as the vehicle (normal saline) treated controls. Group II animals received CP intraperitoneally dissolved in saline, in a dose of 150 mg/kg b.wt., twice, for two consecutive days. Group III animals received SQ orally in a dose of 0.4 ml/day/rat on all the days of the experimental period (12 days) {Various doses of SQ (0.2, 0.4, 0.6, 0.8 and 1.0 ml/day/rat) were administered orally to the CP intoxicated animals to optimize the SQ dose for a maximum efficacy in the minimum dose, determined by the levels of serum marker enzymes for tissue damage (data not shown). It was found that 0.4 ml/day/rat of SQ have the maximum protective efficacy in the minimum dose. This dose of SQ has the protective efficacy against CP induced toxicities in the rats [36, 37]}. Group IV animals were co-treated with CP (as in Group II) and SQ (as in Group III) for the first two days and SQ treatment was followed continuously daily for ten days up to the end of the experimental period.

Subramanian Senthilkumar, Kesavarao Kumar Ebenezar, Venkatachalem Sathish, Surinderkumar Yogeeta, Thiruvengadam Devaki

NS – not significant

Figure 1. Levels of TBARS in the liver of the experimental animals. Results are given as mean ± S.D. for six rats. Comparisons are made between Control (group I) with CP (group II), CP with CP+SQ (group IV) and Control with SQ (group III). Statistical significance: *P≤0.05, **P≤0.01 and ***P≤0.001

At the end of the experimental period, all the animals were sacrificed by cervical decapitation. The liver and kidney were immediately excised and rinsed with ice-cold physiological saline. These were homogenized in 0.1 M Tris-HCl buffer (pH 7.4) and aliquots of this homogenate were used for the assays.

SOD was assayed by the method of Misra and Fridovich [38]. The degree of inhibition of auto oxidation of epinephrine at an alkaline pH by SOD was used as a measure of enzyme activity. CAT was assayed by the method of Takahara et al. [39]. The amount of hydrogen peroxide consumed by the enzyme was used as a measure of the enzyme activity. GPx was assayed by the method of Rotruck et al. [40]. The enzyme activity was assessed in terms of utilization of the glutathione and is based on the remaining glutathione after the reaction, which forms a complex with 5,5-dithio-bis {2-nitrobenzoic acid} (DTNB). GST was assayed by the method of Habig et al. [41]. GR that converts oxidized glutathione (GSSG) to the reduced form (GSH) was assayed by the method of Staal et al.

[42]. GSH was determined by the method of Moron et al. [43]. The level of lipid peroxidation was assayed by the method of Ohkawa et al. [44] and was expressed as nmoles of TBARS g^{-1} of tissue.

Statistical analysis

The results were expressed as the mean ± standard deviation for six animals in each group. Differences between the groups were assessed by the analysis of variance (ANOVA) using the SPSS 10.0 software for Windows. Post-hoc testing was performed using the least significance difference (LSD) test.

Results

Severe biochemical changes in the liver and kidney of the experimental animals were observed due to oxidative damage during the intraperitoneal administration of CP. Table I shows the abnormally decreased activities of enzymic antioxidants in the liver of the experimental animals that indicate cellular damage caused by CP. The activities of SOD, CAT, GPx, GST and GR were decreased by 25.8, 31.7, 32.2, 41.5 and 47.3 %, respectively, in Group II animals when compared with the control (Group I). Activities of these antioxidant enzymes were restored to near normalcy after SQ administration p.o. to the CP toxicated rats. No significant alterations were observed in any of these parameters in the SQ alone (Group III) administered rats except the level of GR, which was significantly elevated than the control.

Toxicity of CP in the liver was also confirmed by the 1.26 fold increase in the level of TBARS (Figure 1) and 45% decrease in the level of GSH (Figure 2) in the group II animals when compared with the control. These abnormal alterations were reverted to significantly normal during the treatment with SQ. No significant changes were observed in the SQ alone administered group, which showed the non-toxic nature of the SQ.

Table II shows the abnormally decreased activities of enzymic antioxidants in the kidney of the

Table I. Activities of enzymic antioxidants in the liver of the experimental animals. Results are expressed as mean ±S.D. for six rats
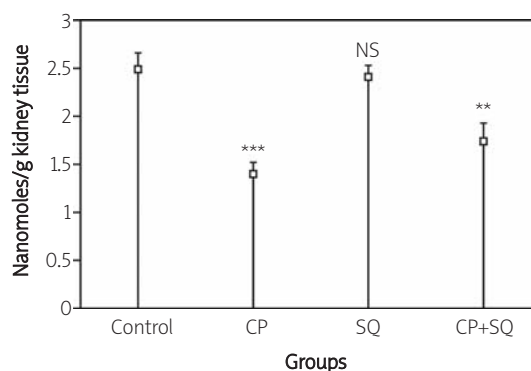
Parameters	Group I (Control)	Group II (CP)	Group III (SQ)	Group IV (CP + SQ)
SOD	9.3±0.57	6.9±0.78***	9.6±1.03[NS]	8.4±0.79**
CAT	65.12±4.64	44.49±5.78***	70.66±8.41[NS]	63.41±8.12***
GPx	103.87±9.44	70.43±8.91***	101.34±7.24[NS]	92.72±8.31**
GST	11.34±0.97	6.63±0.69***	11.47±1.27[NS]	8.35±0.74**
GR	1.65±0.08	0.87±0.07*	1.75±0.07*	1.33±0.12***

*Units – SOD: 50% inhibition of epinephrine auto oxidation min^{-1}; CAT: nmoles of H$_2$O$_2$ decomposed min^{-1} mg^{-1} protein; GPx: nmoles of GSH oxidized min^{-1} mg^{-1} protein; GST: Units min^{-1} mg^{-1} protein; GR: nmoles of NADPH oxidized min^{-1} mg^{-1} protein. Comparisons are made between Control (group I) with CP (group II), CP with CP+SQ (group IV) and Control with SQ (group III). Statistical significance: *P≤0.05, **P≤0.01 and ***P≤0.001*
NS – not significant
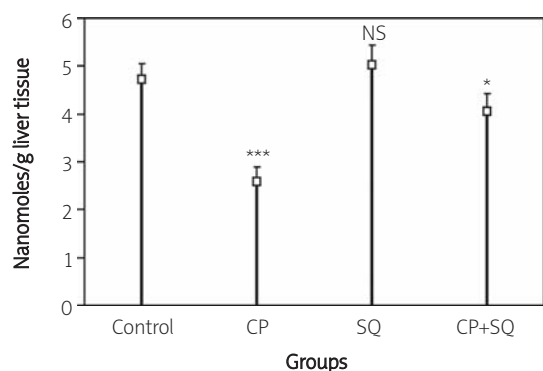
NS – not significant

Figure 2. Levels of GSH in the liver of the experimental animals. Results are given as mean ±S.D. for six rats. Comparisons are made between Control (group I) with CP (group II), CP with CP+SQ (group IV) and Control with SQ (group III). Statistical significance: *P≤0.05, **P≤0.01 and ***P≤0.001
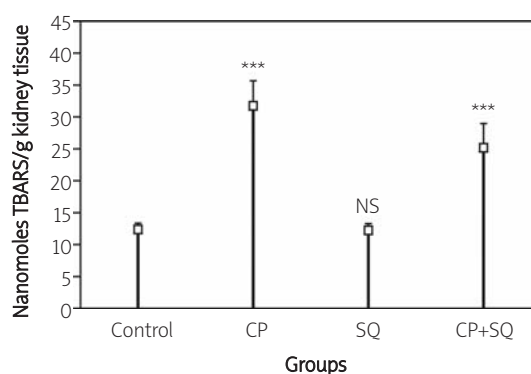

NS – not significant

Figure 4. Levels of GSH in the kidney of the experimental animals. Results are given as mean ±S.D. for six rats. Comparisons are made between Control (group I) with CP (group II), CP with CP+SQ (group IV) and Control with SQ (group III). Statistical significance: *P≤0.05, **P≤0.01 and ***P≤0.001

experimental animals. The activities of SOD, CAT, GPx, GST and GR were decreased by 26.2, 32.4, 25.6, 31.8 and 24.4 %, respectively, in Group II animals when compared with the control (Group I). Activities of these antioxidant enzymes were restored to near normalcy after SQ administration p.o. to the CP toxicated rats. No significant alterations were observed in any of these parameters in the SQ alone (Group III) administered rats. 1.57 fold increase in the level of TBARS (Figure 3) and 43.78% decrease in the level of GSH (Figure 4) in the group II animals when compared with the control also confirm the CP nephrotoxicity. These abnormal alterations were reverted to significantly normal during the treatment with SQ. No significant changes were observed in the SQ alone administered group.

Discussion

High doses of CP can cause an acute form of lethality within 10 days of its administration [45]. In

NS – not significant

Figure 3. Levels of TBARS in the kidney of the experimental animals. Results are given as mean ±S.D. for six rats. Comparisons are made between Control (group I) with CP (group II), CP with CP+SQ (group IV) and Control with SQ (group III). Statistical significance: *P≤0.05, **P≤0.01 and ***P≤0.001

Table II. Activities of enzymic antioxidants in the kidney of the experimental animals. Results are expressed as mean ±S.D. for six rats

Parameters	Group I (control)	Group II (CP)	Group III (SQ)	Group IV (CP + SQ)
SOD	4.35±0.38	3.21±0.42***	4.29±0.35NS	3.94±0.4*
CAT	10.33±1.12	6.98±0.83***	10.38±0.96NS	8.94±0.71**
GPx	57.49±4.91	42.75±5.32***	55.94±6.31NS	55.79±4.4***
GST	9.48±1.04	6.47±0.75***	9.12±0.83NS	8.38±0.94**
GR	0.78±0.09	0.59±0.05**	0.8±0.07NS	0.72±0.08**

*Units – SOD: 50% inhibition of epinephrine auto oxidation min⁻¹; CAT: nmoles of H_2O_2 decomposed min⁻¹ mg⁻¹ protein; GPx: nmoles of GSH oxidized min⁻¹ mg⁻¹ protein; GST: Units min⁻¹ mg⁻¹ protein; GR: nmoles of NADPH oxidized min⁻¹ mg⁻¹ protein. Comparisons are made between Control (group I) with CP (group II), CP with CP+SQ (group IV) and Control with SQ (group III). Statistical significance: *P≤0.05, **P≤0.01 and ***P≤0.001*
NS – not significant

our previous studies, it has been proved that the toxicity induced by CP within 10 days of the CP administration was attenuated by the treatment of SQ [36], evidenced by the significant normalization of clinical chemistry parameters and histopathological studies of the liver and kidney. Administration of intermittent massive doses of CP has been found to be advantageous in the chemotherapy [46]. The cellular mechanism of toxicity is mediated by an increase in the free radicals through intracellular phosphoramide mustard and acrolein, the principle alkylating metabolites of CP [47]. Increased free radical production stimulates lipid peroxidation and is the sources for the degradation of DNA, lipids and carbohydrates [48]. ROS can affect many kinds of proteins, interfering with the enzyme activity and the functions of the structural proteins [49]. The antioxidant enzymes SOD, CAT and GPx act in coordination to combat the formed ROS. Cellular defense against the intermediates of dioxygen reduction (superoxide radical, hydroxyl radical and hydrogen peroxide) were done by these enzymic antioxidants. A decrease in the activities of the antioxidant enzymes of CP administered rats was due to the inactivation of these enzymes by ROS [13]. This causes further elevation in the levels of ROS which severely decrease the activities of SOD, CAT and GPx. This is consistent with the previous report [50]. Yoshiyuki Kohno et al. stated that the rate constant of quenching of singlet oxygen by SQ was similar to that of α-tocopherol [51]. The SQ treated group (Group IV) showed improved activities of SOD, CAT and GPx than the CP group. These evidenced the low ROS level and ROS mediated inactivation of enzymes were prevented by SQ protecting the hepatocytes and nephrocytes from damage.

GST isozymes catalyse the conjugation of glutathione to several electrophilic compounds, including polyaromatic hydrocarbon [52]. CP administration decreased the activities of GSH metabolizing enzymes, GR and GST. GR contains one or more sulphydryl group residues, which are essential for the catalytic activity and are vulnerable to free radicals [53]. Another possible mechanism for the decreased activity of GR could be due to the selective reaction of acrolein with the active site sulfhydryl cysteine [54]. These might be the reason for the decreased activity of GR in CP administration. This, in turn reduces the regeneration of GSH from GSSG by GR. The decreased availability of GSH partly might be responsible for the decreased activity of GST and also because of its oxidative modification in its protein structure. SQ treatment enhanced the activities of GR and GST. This reveals the attenuation of CP toxicity by SQ. It can also be hypothesized that SQ might have provided an additional alkylation site for CP toxic metabolites and exert its protection. The activity of GR was significantly increased in the SQ alone administered group than the control, which could be due to the antioxidant and the membrane stabilizing potential of SQ.

Increased lipid hydroperoxides and thiobarbituric acid-reactive substances also suggest oxidative stress in patients receiving chemotherapy [55]. Pathological changes associated with the significant increase in LPO, depletion in non-protein sulfhydryl groups and decrease in CAT activity reflects many functional alterations such as drop in the glomerular filtration rate, glomerular capillary damage and tubulotoxicity [56]. Due to the lipid peroxidation induced by CP, the levels of TBARS increased in the liver and kidney. This might be due to the increased production of free radicals or decreased enzymic antioxidant defense system. Following the administration of SQ, the levels of TBARS were maintained to the near normal status which indicates the reduced level of LPO. From this, it could be concluded that the oxidative stress induced by CP was attenuated by SQ due to its antioxidant property [29] and membrane stabilizing property [30]. This is consistent with the previous reports of our laboratory [37].

Glutathione, the non-enzymic antioxidant is an important scavenger of electrophiles such as acrolein [40, 57] and has been shown to be a critical factor in the toxicity of CP to hepatocytes [58]. GSH is known to protect renal and neuronal cells from cisplatin induced toxicity [59]. Depletion of plasma and tissue GSH appears to contribute to chemotherapy induced organ toxicity [60, 61]. In the present study, oxidative stress due to CP decreased the GSH levels in the liver and kidney. SQ treatment increased the levels of GSH (might be indirectly by increasing the GR activity), which is essential for detoxification processes and exerts protection for these tissues.

Conclusions

In the present study, the toxicity produced by CP, evidenced by decreased activities of enzymic antioxidants, elevated levels of TBARS and decreased GSH levels in the liver and kidney was attenuated by the treatment of SQ to the experimental rats. From these observations, it is possible to conclude that SQ was found to be effective in normalizing these pathological changes by modulating the antioxidant defense system through enzymic antioxidants.

Reference

1. Oka JM, Simic DV, Simic TP. Free radicals in cardiovascular diseases. Medicine and Biology 1999; 6: 11-22.
2. Bukowski R. The need for cytoprotection. Eur. J. Cancer 1999; 32A: S2- S4.
3. Fraiser LH, Kanekel S, Kehrer JP. Cyclophosphamide toxicity: Characterizing and avoiding the problem. Drugs 1991; 42: 781-795.
4. Dollery C. Cyclophosphamide. In: Dollery C, editor. Therapeutic drugs. Edinburg: Churchill Livingstone; 1999. p. 349-353.

5. Goldberg MA, Antin JH, Guinan EC, Rappeport JM. Cyclophosphamide cardiotoxicity: an analysis of doing as a risk factor. Blood 1986; 68: 1114-1118.

6. Jonas CR, Puckett AB, Jones DP et al. Plasma antioxidant status after high-dose chemotherapy: a randomized trial of parenteral nutrition in bone marrow transplantation patients. Am. J. Clin. Nutr. 2000; 72: 181-189.

7. Ludeman SM. The chemistry of the metabolites of cyclophosphamide. Curr. Pharm. Des. 1999; 5: 627-643.

8. Kern JC, Kehrer JP. Acrolein–induced cell death: a caspase-influenced decision between apoptosis and oncosis/necrosis. Chem.-Biol. Interact. 2002; 139: 79-95.

9. Weijl NI, Leton FJ, Osanto S. Free radicals and antioxidants in chemotherapy-induced toxicity. Cancer Treat. Rev. 1997; 23: 209-240.

10. Shao B, Fu X, McDonald TO et al. Acrolein impairs ATP binding cassette transporter A1-dependent cholesterol export from cells through site-specific modification of apolipoprotein A-1. J. Biol. Chem. 2005; 280: 36386-36396.

11. Witz G. Biological interactions of alpha, beta-unsaturated aldehydes. Free Radic. Biol. Med. 1989; 7: 333-349.

12. Arumugam N, Sivakumar V, Thanislass J, Devaraj H. Effects of acrolein on rat liver antioxidant defense system. Indian J. Exp. Biol. 1997; 35: 1373-1374.

13. Mythili Y, Sudharsan PT, Selvakumar E, Varalakshmi P. Protective effect of DL-α-lipoic acid on cyclophosphamide induced oxidative cardiac injury. Chem.-Biol. Interact. 2004; 151: 13-19.

14. Kawanishi M, Matsuda T, Nakayama A, Takebe H, Matsui S, Yagi T. Molecular analysis of mutations induced by acrolein in human fibroblast cells using supf shuttle vector plasmids. Mut. Res. Gen. Toxicol. Environ. Mutagen. 1998; 417: 65-73.

15. Davies KJA. Protein modification by oxidants and the role of proteolytic enzymes. Biochem. Soc. Trans. 1993; 21: 346-353.

16. Snover DC, Weisdorf S, Bloomer J, McGlave P, Weisdorf D. Nodular regenerative hyperplasia of the liver following bone marrow transplantation. Hepatology 1989; 9: 443-448.

17. Atkinson K, Biggs J, Noble G, Ashby M, Concannon A, Dodds A. Preparative regimens for marrow transplantation containing busulfan are associated with hemorrhagic cystitis and hepatic veno-occlusive disease but a short duration of leucopenia and little oro-pharyngeal mucositis. Bone Marrow Transplant 1987; 2: 385-394.

18. Shulman HM, McDonald GB, Matthews D et al. An analysis of hepatic venocclusive disease and centrilobular hepatic degeneration following bone marrow transplantation. Gastroenterology 1980; 79: 1178-1191.

19. Bearman SI. The syndrome of hepatic veno-occlusive disease after marrow transplantation. Blood 1995; 85: 3005-3020.

20. Rossi R. Nephrotoxicity of ifosfamide – moving towards understanding the molecular mechanisms. Nephrol. Dial Transplant 1997; 12: 1091-1092.

21. Kleta R, Fehring S, Rossi R, Mohrmann M, Schlatter E. Effects of ifosfamide and metabolites on membrane voltage (Vm)/conductance (gm) of LLCPK1 cells. J. Am. Soc. Nephrol. 1996; abstract 7: 1841.

22. Vizzard MA. Alterations in neuropeptide expression in lumbosacral bladder pathways following chronic cystitis. J. Chem. Neuroanal. 2001; 21: 125-138.

23. Yoshimura N, de Groat WC. Increased excitability of afferent neurons innervating rat urinary bladder following chronic bladder inflammation. J. Neurosci. 1999; 19: 4644-4653.

24. Fouladi M, Stempak D, Gammon J et al. Phase I trial of a twice-daily regimen of amifostine with ifosfamide, carboplatin and etoposide chemotherapy in children with refractory carcinoma. Cancer 2001; 92: 914-923.

25. Shaw PJ, Blekley M. Systemic inflammatory response syndrome associated with amifostine. Med. Pediatr. Oncol. 2000; 34: 309-310.

26. Henslay ML, Schuchter LM, Lindley C et al. American Society of clinical oncology clinical practice guidelines for the use of chemotherapy and radiotherapy protectants. J. Clin. Oncol. 1999; 17: 3333-3355.

27. Burcham PC, Pyke SM. Hydralazine inhibits rapid acrolein-induced protein oligomerization: Role of aldehyde scavenging and adduct trapping in cross-link blocking and cytoprotection. Mol. Pharmacol. 2006; 69: 1056-1065.

28. Qureshi AA, Lehmann JW, Peterson DM. Amaranth and its oil inhibit cholesterol biosynthesis in six-week-old female chickens. J. Nutr. 1996; 126: 1972-1978.

29. Ko TF, Weng TM, Chiou RY. Squalene content and antioxidant activity of Terminalia catappa leaves and seeds. J. Agric. Food Chem. 2002; 50: 5343-5348.

30. Ivashkevich SP, Apukhorskaia LI, Vendt VP. Effects of sterols having different chemical structure and squalene on osmotic resistance of erythrocytes. Biokhimiia 1981; 46: 1420-1425.

31. Saint-Leger D, Bague A, Cohen E, Chivot M. A possible role for squalene in the pathogenesis of acne, I, In vitro study of squalene oxidation. Br. J. Dermatol. 1986; 114: 535-542.

32. Storm HM, Oh SY, Kimler BF, Norton S. Radioprotection of mice by dietary squalene. Lipids 1993; 28: 555-559.

33. Fan S, Ho I, Yeoh FL, Lin C, Lee T. Squalene inhibits sodium arsenite-induced sister chromatid exchanges and micronuclei in Chinese hamster ovary-K1 cells. Mutat. Res. 1996; 368: 165-169.

34. Kamimara H, Koga N, Oguri K, Yoshimura H. Enhanced elimination of theophylline, phenobarbital and strychnine from the bodies of rats and mice by squalene treatment. J. Pharmacobiodyn. 1992; 15: 215-221.

35. Richter E, Schafer SG. Effect of squalene on hexachlorobenzene (HCB) concentrations in tissues of mice. J. Environ. Sci. Health B 1982; 17: 195-203.

36. Senthilkumar S, Devaki T, Manohar BM, Babu MS. Effect of squalene on cyclophosphamide induced toxicity. Clin. Chim. Acta 2006; 364: 335-342.

37. Senthilkumar S, Yogeeta SK, Subashini R, Devaki T. Attenuation of cyclophosphamide induced toxicity by squalene in experimental rats. Chem.-Biol. Interact. 2006; 160: 252-260.

38. Misra HP, Fridovich I. The role of superoxide anion in the auto oxidation of epinephrine and a simple assay of superoxide dismutase. J. Biol. Chem. 1972; 247: 3170-3175.

39. Takahara S, Hamilton BH, Nell JV, Kobra TY, Ogura Y, Nishimura ET. Hypocatalasemia, a new genetic carrier state. J. Clin. Invest. 1960; 29: 610-619.

40. Rotruck JT, Pope AL, Gasther HE, Hafeman DG, Hoekstra WG. Selenium biochemical role as a component of glutathione peroxidase. Science 1973; 179: 588-590.

41. Habig WH, Pabst MJ, Jakoby WB. Glutathione S transferases: The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 1974; 249: 7130-7139.

42. Staal GE, Visser J, Veeger C. Purification and properties of glutathione reductase of human erythrocytes. Biochim. Biophys. Acta 1969; 185: 39-48.

43. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S transferase activities in rat lung and liver. Biochim. Biophys. Acta 1979; 582: 67-78.

44. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbutric acid reaction. Anal. Biochem. 1979; 95: 351-358.

45. Gharib MI, Burnett AK. Chemotherapy induced cardiotoxicity: current practice and prospects of prophylaxis. Eur. J. Heart Fail. 2002; 4: 235-242.

46. O'Connel TX, Benenbaum MC. Cardiac and pulmonary effects of high doses of cyclophosphamide and isophosphamide. Cancer Res. 1974; 34: 1586-1591.

47. Lee LK, Harman GS, Hohl RJ, Gingrieh RD. Fatal cyclopho-sphamide cardiomyopathy: its clinical course and treatment. Bone Marrow Transplant 1996; 18: 573-577.

48. Quinlan GJ, Gutteridege JMC. Hydroxyl radical generation by the tetracycline antibodies with free radical damage to DNA, lipids, carbohydrates in the presence of iron and copper salts. Biol. Med. 1988; 5: 341-348.

49. Kopff M, Kowalczyk E, Kopff A. Influence of selected cardiological drugs on oxidative status. Pol. J. Pharmacol. 2004; 56: 265-269.

50. Pigeolet E, Corbisier P, Houbion A et al. Glutathione peroxidase, superoxide dismutase and catalase inactivation by peroxides and oxygen derived free radicals. Mech. Ageing Dev. 1990; 51: 283-297.

51. Kohno Y, Egawa Y, Itoh S, Nagaoka S, Takahashi M, Mukai K. Kinetic study of quenching reaction of singlet oxygen and scavenging reaction of free radical by squalene in n-butanol. Biochim. Biophys. Acta 1995; 1256: 52-56.

52. Peresa FP, Estabrook A, Hewer A et al. Carcinogen-DNA adducts in human breast tissue. Cancer Epidemiol. Biomarkers Prev. 1995; 4: 233-238.

53. Mize CE, Langdon RG. Hepatic glutathione reductase. I. Purification and general kinetic properties. J. Biol. Chem. 1962; 237: 1589-1595.

54. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic. Biol. Med. 1991; 11: 81-128.

55. Clemens MR, Ladner C, Schmidt H et al. Decreased essential antioxidants and increased lipid hydroperoxides following high-dose radiochemotherapy. Free Radic. Res. Commun. 1989; 7: 227-232.

56. Badary OA, Abdel-Naim AB, Abdel-Wahab MH, Hamada FMA. The influence of thymoquinone on doxorubicin-induced hyperlipidemic nephropathy in rats. Toxicology 2000; 143: 219-226.

57. Alarcon RA. Studies on the in vivo formation of acrolein: 3-hydroxypropylmercapturic acid as an index of cyclopho-sphamide (NSC-26271) activation. Cancer Treat. Rep. 1976; 60: 327-335.

58. DeLeve LD. Cellular target of cyclophosphamide toxicity in the murine liver: Role of glutathione and site of metabolic activation. Hepatology 1996; 24: 830-837.

59. Hospers GA, Eisenhauer EA, de Vries EG. The sulfhydryl containing compounds WR-2721 and glutathione as radio- and chemoprotective agents. A review, indications for use and prospects. Br. J. Cancer 1999; 80: 629-638.

60. Shulman HM, Luk K, Deeg HJ, Shuman WB, Storb R. Induction of hepatic veno-occlusive disease in dogs. Am. J. Pathol. 1987; 126: 114-125.

61. Appenroth D, Winnefeld K. Role of glutathione for cisplatin nephrotoxicity in young and adult rats. Ren. Fail. 1993; 15: 135-139.