International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 4 (2): 78-87, 2008
ISSN 1811-7775

Cardioprotective Effect of *Ficus hispida* Linn. on Cyclophosphamide Provoked Oxidative Myocardial Injury in a Rat Model

T.S. Shanmugarajan, M. Arunsundar, I. Somasundaram,
E. Krishnakumar, D. Sivaraman and V. Ravichandiran
Department of Pharmaceutical Biotechnology, Vel's College of Pharmacy,
Velan Nagar, P.V. Vaithiyalingam Road, Pallavaram, Chennai-600117, India

Abstract: The current communication was designed to assess the cardioprotective effect of the methanolic leaf extract of *Ficus hispida* Linn. (FH) (400 mg kg^{-1} body weight, administered orally for 10 days) on cyclophosphamide (CP) provoked oxidative injury in rat heart. CP cardiotoxicity, induced by single intraperitoneal injection (200 mg kg^{-1} b.wt.), was revealed by elevated serum creatine phosphokinase (CPK), lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT). CP induced rats, treated with FH depicted near normalcy in these parameters. In the CP group, increased oxidative stress was evidenced by a significant rise in myocardial malondialdehyde (MDA) level and decline in superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST) and reduced glutathione (GSH) activities in the heart tissue. FH treated rats displayed a significant inhibition of lipid peroxidation (LPO) and augmentation of endogenous antioxidants. These results give credence to the notion that treatment with *F. hispida* leaf extract ameliorates CP induced cardiotoxicity and might serve as a novel combination therapy with CP to combat oxidative stress-mediated myocardial injury.

Key words: *Ficus hispida*, cyclophosphamide, oxidative stress, cardiotoxicity, antioxidants

INTRODUCTION

Cyclophosphamide (CP), a cytotoxic alkylating agent, is extensively used as an antineoplastic agent for the treatment of haematological malignancies and a variety of solid tumours, including leukaemia, ovarian cancer and small-cell lung cancer (Zhang *et al*., 2006) as well as an immunosuppressive agent for organ and bone marrow transplantations (Demirer *et al*., 1996; Itescu *et al*., 2002). Moreover, CP has been widely used as an immunosuppressive agent in the treatment of several autoimmune diseases, including systemic lupus erythematosus (SLE) (Barile-Fabris *et al*., 2005) and rheumatoid arthritis (Verburg *et al*., 2005). Despite its wide spectrum of clinical uses, CP is known to cause multiple organ toxicity (De Souza *et al*., 2000). High therapeutic doses of cyclophosphamide could cause a lethal cardiotoxicity that presents a combination of symptoms and signs of myo-pericarditis which could lead to fatal complications such as congestive heart failure (CHF), arrhythmias and cardiac tamponade (Gharib and Burnett, 2002).

CP itself is a prodrug and it is bioactivated by hepatic cytochrome P450 enzymes via the predominant pathway, 4-hydroxylation (Lindley *et al*., 2002) resulting in the formation of 4-hydroxycyclophosphamide (HCY), the major active circulating metabolite that is converted intracellularly to its tautomer aldophosphamide (Ren and Slattery, 1999). Aldophosphamide is metabolised to phosphoramide mustard (PM) and acrolein (Murgo and Weinberger, 1993). PM brings about interstrand cross-links between opposite DNA strands and hampers the replication and transcription processes that characterises the clinical activity of CP (Dong *et al*., 1995; Paolo *et al*., 2004). Hence, the therapeutic effect of cyclophosphamide is attributed to PM, while the other CP metabolite, acrolein is associated with toxic side effects (Colvin, 1999; Kern and Kehrer, 2002; Pass *et al*., 2005). The cellular mechanism of CP toxicity is due to the production of highly reactive oxygen free radicals by these metabolites (Lee *et al*., 1996). It is obvious that high levels of ROS within the body could culminate in oxidative stress (Scherz-Shouval and Elazar, 2007). In this regard, evidences reveal that oxidative stress plays a key role in the pathogenesis of CP induced cardiotoxicity (Lee *et al*., 1996).

In recent years, the therapeutic strategies are focused on the search of potential drugs of plant origin that

Corresponding Author: T.S. Shanmugarajan, Department of Pharmaceutical Biotechnology, Vel's College of Pharmacy, Velan Nagar, P.V. Vaithiyalingam Road, Pallavaram, Chennai-600117, India
Tel: +91 44 22662500 Fax: +91 44 22662513

possess the ability to minimize the noxious effects induced by chemotherapy to normal cells with out compromising its anti-cancer activity. Plant extracts and natural compounds have also shown protective effect on CP-induced toxicity (Haque *et al*., 2001, 2003; Kumar and Kuttan, 2005; Sharma *et al*., 2000; Sudharsan *et al*., 2005).

The genus *Ficus* constitutes an important group of trees, not only of their immense medicinal value but also of their growth habits and religious significance. The genus *Ficus* is an exceptionally large pantropical genus with over 700 species and belongs to the family Moraceae. *Ficus hispida* Linn., a rough-leaved fig commonly known as Peyatti (Tamil), Dumoor (Bengali) and Gobla (Hindi) is a shrub or moderate sized tree, widely distributed in India and the Andaman Islands in damp localities and in shady places. Almost all parts of this plant are used as a folklore remedy for the treatment of various ailments by Indian traditional healers but the leaves are of particular interest from a medicinal point of view (Nadkarni, 1976), as an anti-diarrhoeal (Mandal and Kumar, 2002), hepatoprotective (Mandal *et al*., 2000), anti-inflammatory (Vishnoi and Jha, 2004), antitussive, antipyretic, astringent, vulnerary, haemostatic and anti-ulcer drug, among other parts (Nadkarni, 1976; Rastogi and Mehrotra, 1993).

The phytochemical constituents of *Ficus hispida* Linn. has not been studied extensively, but the isolation of phenanthroindolizidine alkaloids, n-alkanes, coumarins and triterpenoids from this plant have been documented (Peraza-Sánchez *et al*., 2002). Previous reports show that *Ficus hispida* leaves contain hispidin, oleanolic acid, bergapten, β-amyrin and β-sitosterol (Huong and Trang, 2006; Khan *et al*., 1991) and the bark comprises lupeol acetate, β-sitosterol and β-amyrin acetate (Acharya and Kumar, 1984; Wang and Coviello, 1975). An ample literature suggests that these compounds exhibit significant antioxidant and/or cardioprotective properties (Du and Ko, 2006; Khushbaktova *et al*., 1996; Ng *et al*., 2000; Somova *et al*., 2003; Sudhahar *et al*., 2007; Sudharsan *et al*., 2006; Vivancos and Moreno, 2005). In this light, we hypothesized that *F. hispida* could be evaluated for its cardioprotective effect. The claim that the cardioprotective activity of *F. hispida* resides in the leaves is speculative and has not yet been documented. The present study was designed to investigate the cardioprotective activity of the methanolic leaf extract of *Ficus hispida* on cyclophosphamide induced oxidative cardiac injury in rats.

MATERIALS AND METHODS

Drugs and chemicals: Cyclophosphamide (Ledoxan®) was purchased from Dabur Pharma Limited, New Delhi, India. All other chemicals and solvents used were of the highest purity and analytical grade.

Plant material: The leaves of *F. hispida* Linn. (Moraceae) were collected during the month of February 2007 from the herbal garden of Anna Siddha Hospital and Research Centre, Chennai, India. A voucher specimen (PARC/2007/Vel's/28) was deposited in the Plant Anatomy Research Centre, Pharmacognosy Institute, Chennai, India and was authenticated by Dr. Jayaraman. Then, the leaves were dried under shade and pulverized in a mechanical grinder and stored in a closed container for further use.

Preparation of extract: The powdered leaves were defatted with petroleum ether (B.P. 60-80°C) and then extracted with methanol in a Soxhlet extractor. On evaporation of methanol from the methanol extract *in vacuo*, a greenish coloured residue was obtained (yield 4.7% (w/w) with respect to the dry starting material) and was stored in a desiccator.

Phytochemical screening: On preliminary screening, the methanol extract showed positive reaction for triterpenoids (Noller *et al*., 1942), Shinoda test for flavonoids (Markham, 1982), steroids (Liebermann, 1885), tannins, saponins and alkaloids (Kokate, 1988).

Animal model: The study was conducted on male Wistar rats (150±10 g). Animals were obtained from the Animal House, Vel's College of Pharmacy. The Tamilnadu Dr. M.G.R. Medical University, Chennai, India. Animals were fed with commercially available standard rat pelleted feed (M/s Pranav Agro Industries Ltd., India) under the trade name Amrut rat/mice feed and water was provided *ad libitum*. The animals were deprived of food for 24 h before experimentation but allowed free access to tap water. The rats were housed under conditions of controlled temperature (25±2°C) and were acclimatized to 12 h light: 12 h dark cycles. Experimental animals were used after obtaining prior permission and handled according to the University and institutional legislation as regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Experimental protocol: The experimental animals were randomized into four groups of six rats each as follows:

Group 1: Control rats received normal saline (1 mL kg^{-1} b.wt.), orally for 10 days.

Group 2: Rats were injected intraperitoneally with a single dose of CP (200 mg kg^{-1} b.wt.) dissolved in saline, on the first day of the experimental period.

Group 3: Rats received FH extract by oral gavage (400 mg kg^{-1} b.wt. for 10 days).

Group 4: Rats were administered CP as in Group 2, immediately followed by supplementation with FH extract (400 mg kg^{-1} b.wt.) by oral gavage for 10 consecutive days.

After the 10 days experimental period (i.e., on the 11th day), all the animals were anesthetized and decapitated. Heart tissues were immediately excised and rinsed in ice cold physiological saline. The tissues were homogenized in 0.01 M Tris-HCl buffer (pH 7.4) and aliquots of this homogenate were used for the assays. Blood was collected and serum was separated for analysis of biochemical parameters.

Enzymatic indices of cellular damage: The activity of creatine phosphokinase (CPK) was assayed by the method of Okinaka *et al.* (1964). Lactate dehydrogenase (LDH) was assayed by the method of King (1965a). The method is based on the ability of LDH to form pyruvate in the presence of coenzyme NAD^+. The pyruvate formed was made to react with 2,4-dinitrophenylhydrazine in hydrochloric acid. The hydrazone formed turns into an orange coloured complex in alkaline medium, which was measured at 420 nm. Aspartate transaminase (AST) and alanine transaminase (ALT) were estimated by the method of King (1965b). Protein content was estimated by the method of Lowry *et al.* (1951).

Lipid peroxidation: Tissue lipid peroxide level was determined by the method of Ohkawa *et al.* (1979). The absorbance was measured photometrically at 532 nm and the concentrations were expressed as nmol malonaldehyde (MDA) min/mg/protein.

Antioxidants: SOD was assayed by the method of Misra and Fridovich (1972). The degree of inhibition of auto oxidation of epinephrine at an alkaline pH by SOD was used as a measure of enzyme activity. Catalase (CAT) level was estimated by the method described by Sinha (1972). Glutathione peroxidase (GPx) was assayed by the method of Rotruck *et al.* (1973), based on the reaction between glutathione remaining after the action of GPx and 5,5'-dithio-bis(2-nitro benzoic acid) to form a complex that absorbs maximally at 412 nm. Glutathione-S-transferase (GST) was assayed by the method of Habig *et al.* (1974). Glutathione reductase (GR) that utilizes NADPH to convert oxidised glutathione (GSSG) to the reduced form was assayed by the method of Staal *et al.* (1969). Total reduced glutathione (GSH) was determined by the method of Ellman (1959).

Statistical analysis: The results were expressed as mean±standard deviation (SD) for 6 animals in each group. Differences between groups were assessed by one-way analysis of variance (ANOVA) using the SPSS 13.0 software package for Windows. Post hoc testing was performed for inter-group comparisons using the least significance difference (LSD) test. p-values<0.05 have been considered as statistically significant.

RESULTS

In the present study, intraperitoneal administration of a single dose of CP (200 mg kg^{-1} b.wt.) induced severe biochemical changes as well as oxidative damage in cardiac tissue. There was a significant ($p<0.05$) rise in the levels of diagnostic marker enzymes (CPK, LDH, AST and ALT) in the serum of Group 2 CP administered rats as compared to that of Group 1 control rats (Table 1). The administration of *Ficus hispida* leaf extract to Group 4 animals restored the levels of these enzymes to near normalcy ($p<0.05$) as compared to those Group 2 CP-injected rats. In *F. hispida* alone administered rats (Group 3) versus controls, no significant changes were observed.

In CP administered rats (Group 2), the increase in serum marker enzyme activities was accompanied by concomitant decreased activities ($p<0.05$) of these enzymes in the heart tissue (Table 2), which depict the damage of heart in Group 2 animals. Activities of these enzymes in the cardiac tissue were restored to near normal levels ($p<0.05$) in *F. hispida* treated rats (Group 4). This may be due to the protection offered by *F. hispida* against tissue damage and oxidative stress induced by cyclophosphamide.

Injection of CP induced a significant ($p<0.05$) increase in the level of lipid peroxidation (LPO), measured in terms of MDA (Fig. 2), which was paralleled by significant ($p<0.05$) reduction in the level of GSH (Fig. 1) in the heart tissue of Group 2 animals as compared to normal controls. Glutathione plays an important role in the regulation of variety of cell functions and in cell protection from oxidative injury. Depletion of GSH results in enhanced lipid peroxidation and excessive lipid

Table 1: Effect of cyclophosphamide and *F. hispida* on the activities of cardiac marker enzymes in serum

Groups	CPK (IU L^{-1})	LDH (IU L^{-1})	AST (IU L^{-1})	ALT (IU L^{-1})
1 (Control)	128.17±6.55	265.92±11.95	83.83±4.07	54.87±2.9
2 (CP)	263.83±22.2^{a*}	428.17±37.07^{a*}	276.17±25.33^{a*}	180.67±15.33^{a*}
3 (FH)	125.17±2.93^{NS}	269.76±13.21^{NS}	83.57±2.35^{NS}	53.17±1.47^{NS}
4 (FH + CP)	138.17±8.42^{b*}	278.00±14.21^{b*}	98.33±4.50^{b*}	60.77±1.56^{b*}

Results are expressed as mean±SD for 6 rats. Comparisons are made between: ^{a}Group 1 and 2; ^{b}Group 2 and 4. *Statistically significant ($p<0.05$); NS: Non-significant

Table 2: Effect of cyclophosphamide and *F. hispida* on the activities of cardiac enzymes

Groups	CPK (IU mg^{-1} protein)	LDH (IU mg^{-1} protein)	AST (IU mg^{-1} protein)	ALT (IU mg^{-1} protein)
1 (Control)	22.47±1.12	33.60±1.46	7.09±0.31	6.03±0.19
2 (CP)	7.25±0.49^{a*}	14.83±1.36^{a*}	3.18±0.38^{a*}	2.76±0.11^{a*}
3 (FH)	23.25±0.85^{NS}	35.05±1.33^{NS}	7.45±0.35^{NS}	5.89±0.41^{NS}
4 (FH + CP)	17.94±0.94^{b*}	28.57±1.7^{b*}	7.03±0.63^{b*}	4.94±0.17^{b*}

Results are expressed as mean±SD for 6 rats. Comparisons are made between: ^{a}Group 1 and 2; ^{b}Group 2 and 4. *Statistically significant ($p<0.05$); NS: Non-significant

Table 3: Effect of cyclophosphamide and *F. hispida* on the activities of cardiac enzymic antioxidants

Groups	SOD (Units mg^{-1} protein)	CAT (µmoles H_2O_2 consumed min^{-1} mg^{-1} protein)	GPx (µmoles min^{-1} mg^{-1} protein)	GST (nmoles min^{-1} mg^{-1} protein)	GR (nmoles min^{-1} mg^{-1} protein)
1 (Control)	5.15±0.11	32.06±0.41	1.86±0.03	0.82±0.04	1.49±0.06
2 (CP)	2.58±0.08^{a*}	18.69±0.42^{a*}	0.83±0.04^{a*}	0.43±0.02^{a*}	0.72±0.03^{a*}
3 (FH)	5.07±0.04^{NS}	33.27±0.21^{NS}	1.91±0.06^{NS}	0.83±0.09^{NS}	1.54±0.03^{NS}
4 (FH + CP)	4.88±0.06^{b*}	28.19±0.57^{b*}	1.71±0.07^{b*}	0.77±0.02^{b*}	1.47±0.05^{b*}

Results are expressed as mean±SD for six rats. Units-SOD: Units mg^{-1} protein, one unit is equal to the amount of enzyme that inhibits auto-oxidation of epinephrine by 50%; CAT: µmoles H_2O_2 consumed min^{-1} mg^{-1} protein; GPx: µmoles GSH oxidized min^{-1} mg^{-1} protein; GST: nmoles CDNB (1-chloro-2,4-dinitrobenzene) conjugated min^{-1} mg^{-1} protein; GR: nmoles NADPH oxidized min^{-1} mg^{-1} protein. Comparisons are made between: ^{a}Group 1 and 2; ^{b}Group 2 and 4. *Statistically significant ($p<0.05$); NS: Non-significant

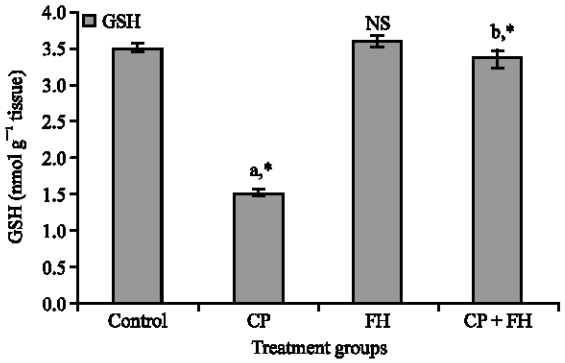

Fig. 1: Levels of GSH in the heart of the experimental animals. Results are given as mean±SD for 6 rats. Comparisons are made between: a-Group 1 and 2; b-Group 2 and 4. *Statistically significant ($p<0.05$); NS: Non-significant

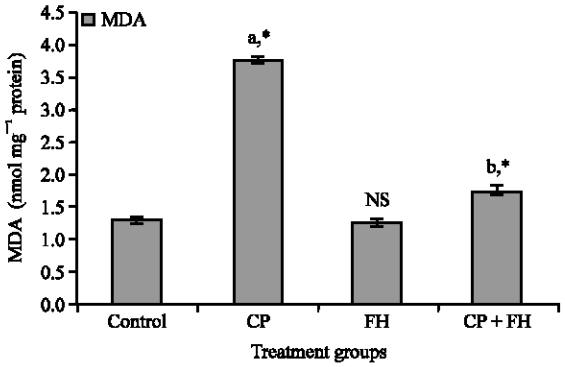

Fig. 2: Levels of MDA in the heart of the experimental animals. Results are given as mean±SD for 6 rats. Comparisons are made between: a-Group 1 and 2; b-Group 2 and 4.*Statistically significant ($p<0.05$); NS: Non-significant

peroxidation can cause increased GSH consumption (Comporti, 1985), as observed in the present study. In this study, the treatment with *F. hispida* (Group 4) significantly ($p<0.05$) counteracted the CP-induced lipid peroxidation and restored the level of GSH to near normal level in Group 4 rats as compared to that of Group 2 animals.

Activities of glutathione-dependent antioxidant enzymes (GPx, GST and GR) and anti-peroxidative enzymes (SOD and CAT) were significantly ($p<0.05$) lower in the heart tissue of Group 2 CP-injected rats as compared to that of Group 1 normal control rats (Table 3). The observed reduction in the activities of GPx, GR and GST in CP-induced myocardial damage might be due to decreased availability of their substrate, reduced glutathione (GSH). In the present study, the treatment of Group 4 rats with *F. hispida*, significantly ($p<0.05$) reversed all these CP-induced alterations in the activities of antioxidant enzymes (SOD, CAT, GPx, GST and GR) to a near normal status. The normal rats receiving *F. hispida*

alone (Group 3) did not show any significant change when compared with control rats, indicating that it does not *per se* have any adverse effects.

DISCUSSION

High-dose cyclophosphamide was introduced as a mainstay of numerous preparative regimens for haemopoietic stem-cell transplantation and its potential to cause myocardial damage was soon recognized. Santos *et al.* (1971) reported the first human fatality of cyclophosphamide (CP) cardiotoxicity as a complication of bone marrow transplantation. Several studies implicate that high-dose cyclophosphamide is associated with cardiotoxicity (Friedman *et al.*, 1990; Goldberg *et al.*, 1986; Zver *et al.*, 2007). The pharmacokinetics and metabolism of CP have been extensively studied (Zhang *et al.*, 2005). CP requires bioactivation to form 4-hydroxy-CP and also aldophosphamide, which spontaneously degrades by β-elimination, to form stoichiometric amounts of phosphoramide mustard and the toxic by-product acrolein (Zon *et al.*, 1984). Acrolein is a highly reactive α, β-unsaturated aldehyde and its formation from CP was first demonstrated by Alarcon and Meienhofer (Gurtoo *et al.*, 1981).

The aetiopathogenesis of CP induced cardiotoxicity is not yet fully unraveled. However, toxicity of CP was postulated to be mediated by oxidative stress (Lee *et al.*, 1996) which may have deleterious effects on the heart. Moreover, it is thought to involve direct endothelial damage, with extravasation of plasma proteins, high concentration of cyclophosphamide and erythrocytes into the myocardial interstitium and muscle cells, resulting in damage of myocardial cells. (Appelbaum *et al.*, 1976; Fraiser *et al.*, 1991). Due to the damage, the enzymes (CPK, LDH, AST and ALT) leak from the necrotic heart cells to the serum, which are important measures of cardiac injury. These enzymes are not specific for myocardial injury individually; however, evaluation of these enzymes together may be an indicator of myocardial injury (Al-Shabanah *et al.*, 1998; Chopra *et al.*, 1995). In CP-administered rats, the activities of these marker enzymes were elevated in serum with a concomitant decrease in the heart tissue. FH treated rats showed near normalcy in these enzyme levels. This might be attributed to the membrane stabilizing effect of the phytoconstituents like oleanolic acid and β-sitosterol, present in the FH (Senthil *et al.*, 2007; Yokota *et al.*, 2006).

Reactive Oxygen Species (ROS) include superoxide anion, hydroxyl radical, alkoxyl radical, peroxyl radical, hydrogen peroxide and singlet oxygen (Halliwell *et al.*, 1995; Simon *et al.*, 2000). Superoxide anion itself is not a strong oxidant, but it reacts with protons in water solution to form hydrogen peroxide (H_2O_2), which can serve as a substrate for the generation of hydroxyl radicals and singlet oxygen (Stief, 2003). The prevalent free radical states, or so-called oxidative stress, initiate the oxidation of polyunsaturated fatty acids (PUFA), proteins, DNA and sterols. Free radicals generated through cyclophosphamide metabolism, cause membrane damage by initiating LPO which leads to impairment in the integrity and function of myocardial membranes. The obtained data reveal that CP exposure produced a marked oxidative impact as reflected by elevated LPO, measured in terms of MDA level in the heart tissue. FH treated rats showed decreased MDA level, due to significant inhibition of LPO which is in line with earlier studies (Mandal *et al.*, 2000). This might be due to the presence of oleanolic acid, hispidin and β-sitosterol which have been reported to possess anti-lipid peroxidation and/or free radical scavenging properties (Liu *et al.*, 1995; Park *et al.*, 2004; Yokota *et al.*, 2006).

Cells are equipped with an impressive repertoire of antioxidant defensive system (Fang *et al.*, 2002). The present study shows that the free radical-induced increase in LPO is accompanied by concomitant decline in the activities of cellular antioxidants. This may be due to the inactivation of cellular antioxidants by lipid peroxides and ROS (Halliwell and Gutteridge, 1984). SOD is inhibited by hydrogen peroxide (H_2O_2) while GPx and CAT by an excess of superoxide radical (Pigeolet *et al.*, 1990). In fact, the heart has a greater susceptibility to oxidative stress than other tissues due to its inherent decreased detoxifying antioxidants (Doroshow *et al.*, 1980; Gustafson *et al.*, 1993). The decrease in endogenous antioxidant enzymes might predispose the cardiac tissue to increased free radical damage, because SOD catalyzes the dismutation of superoxide anion to hydrogen peroxide, while CAT and GPx are involved in cellular detoxification and can convert H_2O_2 into water and oxygen (Konorev *et al.*, 1999). GPx is the most important hydrogen peroxide-removing enzyme existing in the membrane. If the activity of CAT or GPx is not adequate to degrade H_2O_2, more H_2O_2 could be converted to toxic hydroxyl radicals and may contribute to the CP-induced oxidative stress. Administration of FH replenished the antioxidant levels, which might be attributed to the free radical scavenging/antioxidant properties of its phytoconstituents described elsewhere in this report.

Reduced glutathione (GSH), the first line of defense against ROS, is a readily available source of endogenous sulfhydryl (-SH) groups. CP exposure caused a dramatic decline in GSH level, which may be ascribed to the direct conjugation of CP's metabolites with free or protein

bound -SH groups (Yuan *et al*., 1991; Yousefipour *et al*., 2005), thereby interfering with the antioxidant functions. The activities of CAT, SOD and GPx were significantly reduced in GSH depleted condition due to pronounced oxidative stress and accumulation of H_2O_2, making the cells more vulnerable to oxidative stress (Rajasekaran *et al*., 2002). FH treatment restored the GSH level to near normalcy. One of the reasons for this restorative effect might be the presence of triterpenoid constituents in FH (Liu *et al*., 1993, 1995; Oliveira *et al*., 2005).

CP treated rats displayed decreased activities of GSH metabolizing enzymes, GST and GR which is consistent with the previous report (Senthilkumar *et al*., 2006). Many investigators have suggested that GST offers protection against LPO by promoting the conjugation of toxic electrophiles with GSH (Jakoby, 1988). GR is a flavoprotein that permits the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH) by the oxidation of NADH to NAD^+ (Papas, 1999). Inactivation of GR in the heart, leads to accumulation of GSSG (Ferrari *et al*., 1985) which in turn inactivates enzymes containing -SH groups and inhibits protein synthesis (Ji *et al*., 1988). Impairment of these enzyme activities may thus be doubly detrimental to the myocardial tissue. *F. hispida* treatment restored the normal activities of these enzymes, thereby confirming its protective action.

Previous studies suggest that oleanolic acid has protective effect against cyclophosphamide-induced toxicities (Liu, 1995). Recent evidence suggests that oleanolic acid has a significant cardioprotective effect (Senthil *et al*., 2007). Intriguingly, literature citations show that phytosterols like β-sitosterol exert antioxidant, cardioprotective properties (Higgs, 2003; Yoshida and Niki, 2003). A recent report suggests the possibility of GSH replenishing effect of β-amyrin (Oliveira *et al*., 2005). Prodigious amounts of literature data suggest that triterpenoids, flavonoids, tannins possess significant antioxidant/cardioprotective effects (Augusti *et al*., 2005; Daniel *et al*., 2003; Hertog *et al*., 1993; Hong *et al*., 1995; Pawar and Bhutani, 2005). Hence, it is suggested that presence of the aforementioned active ingredients in *F. hispida* leaf extract might be responsible for the abrogation of CP elicited cardiotoxicity.

CONCLUSION

To summarize, the results of the present study indicate that cyclophosphamide exposure results in the pronounced oxidative stress and tissue damage. Administration of *Ficus hispida* leaf extract protects the cardiac tissue by scavenging the free radicals, which is evidenced by the normalization of the biochemical parameters. These observations support the hypothesis that *Ficus hispida* has potential for its evaluation as a cardioprotective agent against CP-induced oxidative myocardial injury. Further studies for the protective role of *Ficus hispida* in cyclophosphamide-induced toxicities are currently under investigation.

REFERENCES

Acharya, B.M. and K.A. Kumar, 1984. Chemical examination of the bark of *Ficus hispida* Linn. Curr. Sci., 53 (19): 1034-1035.

Al-Shabanah, O., M. Mansour, H. El-Kashef and A. Al-Bekairi, 1998. Captopril ameliorates myocardial and hematological toxicities induced by adriamycin. Biochem. Mol. Biol. Int., 45 (2): 419-427.

Appelbaum, F.R., J.A. Strauchen, Jr. R.G. Graw, D.D. Savage, K.M. Kent, V.J. Ferrans and G.P. Herzig, 1976. Acute lethal carditis caused by high-dose combination chemotherapy: A unique clinical and pathological entity. Lancet, 1 (7950): 58- 62.

Augusti, K.T., Anuradha, S.P. Prabha, K.B. Smitha, M. Sudheesh, A. George and M.C. Joseph, 2005. Nutraceutical effects of garlic oil, its nonpolar fraction and a *Ficus* flavonoid as compared to vitamin E in CCl_4 induced liver damage in rats. Indian J. Exp. Biol., 43 (5): 437-444.

Barile-Fabris, L., R. Ariza-Andraca, L. Olguin-Ortega, L.J. Jara, A. Fraga-Mouret, J.M. Miranda-Limon, J.F. Mata, P. Clark, F. Vargas and J. Alocer-Varela, 2005. Controlled clinical trial of IV cyclophosphamide versus IV methylprednisolone in severe neurological manifestations in systemic lupus erythematosus. Ann. Rheum. Dis., 64 (4): 620-625.

Chopra, S., K.K. Pillai, S.Z. Husain and D.K. Giri, 1995. Propolis protects against doxorubicin-induced myocardiopathy in rats. Exp. Mol. Pathol., 62 (3): 190-198.

Colvin, O.M., 1999. An overview of cyclophosphamide development and clinical applications. Curr. Pharm. Des., 5 (8): 555-560.

Comporti, M., 1985. Lipid peroxidation and cellular damage in toxic liver injury. Lab. Invest., 53 (6): 599-623.

Daniel, R.S., K.S. Devi, K.T. Augusti and C.R.S. Nair, 2003. Mechanism of action of antiatherogenic and related effects of *Ficus bengalensis* Linn. flavonoids in experimental animals. Indian J. Exp. Biol., 41 (4): 296-303.

Demirer, T., C.D. Buckner, F.R. Appelbaum, W.I. Bensinger, J. Sanders, K. Lambert, R. Clift, A. Fefer, R. Storb and J.T. Slattery, 1996. Busulfan, cyclophosphamide and fractionated total body irradiation for autologous or syngeneic marrow transplantation for acute and chronic myelogenous leukemia: Phase I dose escalation of busulfan based on targeted plasma levels. Bone Marrow Transplant., 17 (4): 491-495.

De Souza, C.A., G. Santini, G. Marino, S. Nati, A.M. Congiu, A.C. Vigorito and E. Damasio, 2000. Amifostine (WR-2721), a cytoprotective agent during high-dose cyclophosphamide treatment of non-Hodgkin's lymphomas: A phase II study. Braz. J. Med. Biol. Res., 33 (7): 791-798.

Dong, Q., D. Barsky, M.E. Colvin, C.F. Melius, S.M. Ludeman, J.F. Moravek, O.M. Colvin, D.D. Bigner, P. Modrich and H.S. Friedman, 1995. A structural basis for a phosphoramide mustard-induced DNA interstrand cross-link at 5'-d(GAC). Proc. Natl. Acad. Sci., USA., 92 (26): 12170-12174.

Doroshow, J.H., G.Y. Locker and C.E. Myers, 1980. Enzymatic defenses of the mouse heart against reactive oxygen metabolites: Alterations produced by doxorubicin. J. Clin. Invest., 65 (1): 128-135.

Du, Y. and K.M. Ko, 2006. Oleanolic acid protects against myocardial ischemia-reperfusion injury by enhancing mitochondrial antioxidant mechanism mediated by glutathione and α-tocopherol in rats. Planta Med., 72 (3): 222-227.

Ellman, G.L., 1959. Tissue sulfydryl groups. Arch. Biochem. Biophys., 82 (1): 70-71.

Fang, Y.Z., S. Yang and G. Wu, 2002. Free radicals, antioxidant and nutrition. Nutrition, 18 (10): 872-879.

Ferrari, R., C. Ceconi, S. Curello, C.M. Guarnieri, A. Albertini and D. Visioli, 1985. Oxygen mediated myocardial damage using ischemia and reperfusion. Role of cellular defences against oxygen toxicity. J. Mol. Cell Cardiol., 17 (10): 937-945.

Fraiser, L.H., S. Kanekel and J.P. Kehrer, 1991. Cyclophosphamide toxicity: Characterizing and avoiding the problem. Drugs, 42 (5): 781-795.

Friedman, H.S., O.M. Colvin, K. Aisaka, J. Popp, E.H. Bossen, K.A. Reimer, J.B. Powell, J. Hilton, S.S. Gross, R. Levi, D.D. Bigner and O.W. Griffith, 1990. Glutathione protects cardiac and skeletal muscle from cyclophosphamide-induced toxicity. Cancer Res., 50 (8): 2455-2462.

Gharib, M.I. and A.K. Burnett, 2002. Chemotherapy-induced cardiotoxicity: Current practice and prospects of prophylaxis. Eur. J. Heart Fail., 4 (3): 235-242.

Goldberg, M.A., J.H. Antin, E.C. Guinan and J.M. Rappeport, 1986. Cyclophosphamide cardiotoxicity: An analysis of doing as a risk factor. Blood, 68 (5): 1114-1118.

Gurtoo, H.L., J.H. Hipkens and S.D. Sharma, 1981. Role of glutathione in the metabolism-dependent toxicity and chemotherapy of cyclophosphamide. Cancer Res., 41 (91): 3584-3591.

Gustafson, D.L., J.D. Swanson and C.A. Pritsos, 1993. Modulation of glutathione and glutathione dependent antioxidant enzymes in mouse heart following doxorubicin therapy. Free Radic. Res. Commun., 19 (2): 111-120.

Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione-S-transferases: The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249 (22): 7130-7139.

Halliwell, B. and J.M. Gutteridge, 1984. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem. J., 219 (1): 1-14.

Halliwell, B., M.A. Murcia, S. Chirico and O.I. Aruoma, 1995. Free radicals and antioxidants in food and *in vivo*: What they do and how they work. Crit. Rev. Food Sci. Nutr., 35 (1-2): 7-20.

Haque, R., B. Bin-Hafeez, I. Ahmad, S. Parvez, S. Pandey and S. Raisuddin, 2001. Protective effects of *Emblica officinalis* Gaertn. in cyclophosphamide-treated mice. Hum. Exp. Toxicol., 20 (12): 643-650.

Haque, R., B. Bin-Hafeez, S. Parvez, S. Pandey, I. Sayeed, M. Ali and S. Raisuddin, 2003. Aqueous extract of walnut (*Juglans regia* L.) protects mice against cyclophosphamide induced biochemical toxicity. Hum. Exp. Toxicol., 22 (9): 473-480.

Hertog, M.G.L., E.J.M. Feskens, P.C.H. Hollman, M.B. Katan and D. Kromhout, 1993. Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen Elderly Study. Lancet, 342 (8878): 1007-1011.

Higgs, J., 2003. The beneficial role of peanuts in the diet Part 2. Nutr. Food Sci., 33 (2): 56-64.

Hong, C.Y., C.P. Wang, S.S. Huang and F.L. Hsu, 1995. The inhibitory effect of tannins on lipid peroxidation of rat heart mitochondria. J. Pharm. Pharmacol., 47 (2): 138-142.

Huong, V.N. and V.M. Trang, 2006. Hispidin. A strong anticancer agent isolated from the leaves of *Ficus hispida* L., Tap. Chi. Hoa. Hoc., 44 (3): 345-349.

Itescu, S., E. Burke, K. Lietz, R. John, D. Mancini, R. Michler, E. Rose, M. Oz and N. Edwards, 2002. Intravenous pulse administration of cyclophosphamide is an effective and safe treatment for sensitized cardiac allograft recipients. Circulation, 105 (10): 1214-1219.

Jakoby, W.B., 1988. Detoxification, Conjugation and Hydrolysis in Liver Biology and Pathology. Arias, I.M. and W.B. Jakoby (Eds.). Raven Press, New York, pp: 375-385.

Ji, L.L., F.W. Stratman and H.A. Lardy, 1988. Antioxidant enzyme systems in rat liver and skeletal muscle. Arch. Biochem. Biophys., 263 (1): 150-160.

Kern, J.C. and J.P. Kehrer, 2002. Acrolein-induced cell death: A caspase-influenced decision between apoptosis and oncosis/necrosis. Chem. Biol. Interact., 139 (1): 79-95.

Khan, M.S.Y., A.A. Siddiqui and K. Javed, 1991. Chemical investigation of the leaves of *Ficus hispida*. Indian J. Nat. Prod., 6 (2): 14-15.

Khushbaktova, Z.A., S.M. Yusupova, K.L. Badal'yants, V.N. Syrov and ÉKh. Batirov, 1996. Isolation of hispidin from a walnut-tree fungus and its antioxidant activity. Chem. Nat. Comp., 32 (1): 27-29.

King, J., 1965a. The Dehydrogenases or Oxidoreductases-Lactacte Dehydrogenase. In: Practical Clinical Enzymology, Van, D. (Ed.). Nostrand Company Limited, London, pp: 83-93.

King, J., 1965b. The Transferases-Alanine and Aspartate Transaminases. In: Practical Clinical Enzymology, Van, D., (Ed.). Nostrand Company Limited, London, pp: 121-138.

Kokate, C.K., 1988. Practical Pharmacognosy. 2nd Edn. Vallabh Prakashan, Delhi, pp: 119-125.

Konorev, E.A., M.C. Kennedy and B. Kalyanaraman, 1999. Cell-permeable superoxide dismutase and glutathione peroxidase mimetics afford superior protection against doxorubicin-induced cardiotoxicity: The role of reactive oxygen and nitrogen intermediates. Arch. Biochem. Biophys., 368 (2): 421-428.

Kumar, K.B.H. and R. Kuttan, 2005. Chemoprotective activity of an extract of *Phyllanthus amarus* against cyclophosphamide induced toxicity in mice. Phytomedicine, 12 (6-7): 494-500.

Lee, L.K., G.S. Harman, R.J. Hohl and R.D. Gingrieh, 1996. Fatal cyclophosphamide cardiomyopathy: Its clinical course and treatment. Bone Marrow Transplant., 18 (3): 573-577.

Liebermann, C., 1885. Uber das Oxychinoferben. Berichte, 18: 1803-1809.

Lindley, C.M., G. Hamilton, J.S. McCune, S. Faucette, S.S. Shord, R.L. Hawke, H. Wang, D. Gilbert, S. Jolley, B. Yan and E.L. Lecluyse, 2002. The effect of cyclophosphamide with and without dexamethasone on cytochrome P450 3A4 and 2B6 in human hepatocytes. Drug Metab. Dispos., 30 (7): 814-822.

Liu, J., Y.P. Liu, C. Madhu and C.D. Klaassen, 1993. Protective effects of oleanolic acid on acetaminophen hepatotoxicity in mice. J. Pharmacol. Exp. Ther., 266 (3): 1607-1613.

Liu, J., 1995. Pharmacology of oleanolic acid and ursolic acid. J. Ethnopharmacol., 49 (2): 57-68.

Liu, J., Y.P. Liu, A. Parkinson and C.D. Klaassen, 1995. Effect of oleanolic acid on hepatic toxicant-activating and detoxifying systems in mice. J. Pharmacol. Exp. Ther., 275 (2): 768-774.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193 (1): 265-275.

Mandal, S.C., B. Saraswathi, C.K.A. Kumar, S.M. Lakshmi and B.C. Maiti, 2000. Protective effect of leaf extract of *Ficus hispida* Linn. against paracetamol-induced hepatotoxicity in rats. Phytother. Res., 14 (6): 457-459.

Mandal, S.C. and C.K.A. Kumar, 2002. Studies on anti-diarrhoeal activity of *Ficus hispida*. leaf extract in rats. Fitoterapia, 73 (7-8): 663-667.

Markham, K.R., 1982. Technique of Flavonoid Identification. Academic Press, New York, pp: 1-113.

Misra, H.P. and I. Fridovich, 1972. The role of superoxide anion in the auto-oxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem., 247 (10): 3170-3175.

Murgo, A.J. and B.B. Weinberger, 1993. Pharmacological bone marrow purging in autologous transplantation: Focus on the cyclophosphamide derivatives. Crit. Rev. Oncol. Hematol., 14 (1): 41-60.

Nadkarni, A.K., 1976. Indian Materia Medica. Popular Prakashan, Bombay, I: 1031-1035.

Ng, T.B., F. Liu and Z.T. Wang, 2000. Antioxidative activity of natural products from plants. Life Sci., 66 (8): 709-723.

Noller, C.R., R.A. Smith, G.R. Harris and J.W. Walker, 1942. Saponins and sapogenins. XX. Some color reactions of triterpenoid sapogenins. J. Am. Chem. Soc., 64 (12): 3047-3049.

Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. Anal. Biochem., 95 (2): 351-358.

Okinaka, S., H. Sugita, H. Mamoi, Y. Toyukura, T. Watanabe, F. Ebashi and S. Ebashi, 1964. Cysteine-stimulated serum creatine kinase in health and disease. J. Lab. Clin. Med., 64: 299-305.

Oliveira, F.A., M.H. Chaves, F.R.C. Almeida, R.C.P. Lima Jr., R.M. Silva, J.L. Maia, G.A.A.C. Brito, F.A. Santos and V.S. Rao, 2005. Protective effect of α and β-amyrin, a triterpene mixture from *Protium heptaphyllum* (Aubl.) March. trunk wood resin, against acetaminophen-induced liver injury in mice. J. Ethnopharmacol., 98 (1-2): 103-108.

Paolo, A.D., R. Danesi and M.D. Tacca, 2004. Pharmacogenetics of neoplastic diseases: New trends. Pharmacol. Res., 49 (4): 331-342.

Papas, A.M., 1999. Other Antioxidants. In: Antioxidant Status, Diet, Nutrition and Health, Papas, A.M. (Ed.). Boca Raton, Fla., CRC Press, pp: 231-248.

Park, I.H., S.K. Chung, K.B. Lee, Y.C. Yoo, S.K. Kim, G.S. Kim and K.S. Song, 2004. An antioxidant hispidin from the mycelial cultures of *Phellinus linteus*. Arch. Pharm. Res., 27 (6): 615-618.

Pass, G.J., D. Carrie, M. Boylan, S. Lorimore, E. Wright, B. Houston, C.J. Henderson and C.R. Wolf, 2005. Role of hepatic cytochrome P450s in the pharmacokinetics and toxicity of cyclophosphamide: Studies with the hepatic cytochrome P450 reductase null mouse. Cancer Res., 65 (10): 4211-4217.

Pawar, R.S. and K.K. Bhutani, 2005. Effect of oleanane triterpenoids from *Terminalia arjuna*-a cardioprotective drug on the process of respiratory oxyburst. Phytomedicine, 12 (5): 391-393.

Peraza-Sánchez, S.R., H.B. Chai, Y.G. Shin, T. Santisuk, V. Reutrakul, N.R. Farnsworth, G.A. Cordell, J.M. Pezzuto and A.D. Kinghorn, 2002. Constituents of the leaves and twigs of *Ficus hispida*. Planta Med., 68 (2): 186-188.

Pigeolet, E., P. Corbisier, A. Houbion, D. Lambert, C. Michiels, M. Raes, M.D. Zachary and J. Remacle, 1990. Glutathione peroxidase, superoxide dismutase and catalase inactivation by peroxides and oxygen derived free radicals. Mech. Ageing Dev., 51 (3): 283-297.

Rajasekaran, N.S., H. Devaraj and S.N. Devaraj, 2002. The effect of glutathione monoester (GME) on glutathione (GSH) depleted rat liver. J. Nutr. Biochem., 13 (5): 302-306.

Rastogi and B.N. Mehrotra, 1993. Compendium of Indian Medicinal Plants, Central Drug Research Institute, Lucknow. Publication and Information Directorate, New Delhi, II: 27-30.

Ren, S. and J.T. Slattery, 1999. Inhibition of Carboxyethylphosphoramide mustard formation from 4-hydroxycyclophosphamide by carmustine. AAPS. Pharm. Sci., 1 (3): 1-8.

Rotruck, J.T., A.L. Pope, H.E. Ganther, A.B. Swanson, D.G. Hafeman and W.G. Hoekstra, 1973. Selenium: biochemical role as a component of glutathione peroxidase. Science, 179 (73): 588-590.

Santos, G.W., L.L. Sensenbrenner, P.J. Burke, M. Colvin, A.H. Jr. Owens, W.B. Bias and R.E. Slavin, 1971. Marrow transplantation in man following cyclophosphamide. Transplant Proc., 3 (1): 400-404.

Scherz-Shouval, R. and Z. Elazar, 2007. ROS, mitochondria and the regulation of autophagy. Trends Cell Biol., 17 (9): 422-427.

Senthil, S., M. Sridevi and K.V. Pugalendi, 2007. Cardioprotective effect of oleanolic acid on isoproterenol-induced myocardial ischemia in rats. Toxicol. Pathol., 35 (3): 418-423.

Senthilkumar, S., S.K. Yogeeta, R. Subashini and T. Devaki, 2006. Attenuation of cyclophosphamide induced toxicity by squalene in experimental rats. Chem. Biol. Interact., 160 (3): 252-260.

Sharma, N., P. Trikha, M. Athar and S. Raisuddin, 2000. Inhibitory effect of *Emblica officinalis* on the *in vivo* clastogenicity of benzo[a]pyrene and cyclophosphamide in mice. Hum. Exp. Toxicol., 19 (6): 377-384.

Simon, H.U., A. Haj-Yehia and F. Levi-Schaffer, 2000. Role of Reactive Oxygen Species (ROS) in the apoptosis induction. Apoptosis, 5 (5): 415-418.

Sinha, A.K., 1972. Colorimetric assay of catalase. Anal. Biochem., 47 (2): 389-394.

Somova, L.I., F.O. Shode, P. Ramnanan and A. Nadar, 2003. Antihypertensive, antiatherosclerotic and antioxidant activity of triterpenoids isolated from *Olea europaea*, subspecies africana leaves. J. Ethnopharmacol., 84 (2-3): 299-305.

Staal, G.E., J. Visser and C. Veeger, 1969. Purification and properties of glutathione reductase of human erythrocytes. Biochim. Biophys. Acta, 185 (1): 39-48.

Stief, T.W., 2003. The physiology and pharmacology of singlet oxygen. Med. Hypotheses, 60 (4): 567-572.

Sudhahar, V., S.A. Kumar, P.T. Sudharsan and P. Varalakshmi, 2007. Protective effect of lupeol and its ester on cardiac abnormalities in experimental hypercholesterolemia. Vascul. Pharmacol., 46 (6): 412-418.

Sudharsan, P.T., Y. Mythili, E. Selvakumar, P. Varalakshmi, 2005. Cardioprotective effect of pentacyclic triterpene, lupeol and its ester on cyclophosphamide-induced oxidative stress. Hum. Exp. Toxicol., 24 (6): 313-318.

Sudharsan, P.T., Y. Mythili, E. Selvakumar and P. Varalakshmi, 2006. Lupeol and its ester ameliorate the cyclophosphamide provoked cardiac lysosomal damage studied in rat. Mol. Cell. Biochem., 282 (1-2): 23-29.

Verburg, R.J., J.K. Sont and J.M. Van-Laar, 2005. Reduction of joint damage in severe rheumatoid arthritis by high-dose chemotherapy and autologous stem cell transplantation. Arthritis Rheum., 52 (2): 421-424.

Vishnoi, S.P. and T. Jha, 2004. Evaluation of anti-inflammatory activity of leaf extracts of *Ficus hispida*. Indian J. Nat. Prod., 20 (3): 27-29.

Vivancos, M. and J.J. Moreno, 2005. Beta-sitosterol modulates antioxidant enzyme response in RAW 264.7 macrophages. Free Radic. Biol. Med., 39 (1): 91-97.

Wang, S. and D.A. Coviello, 1975. The isolation, characterization and synthesis of 10-ketotetracosyl arachidate from *Ficus hispida*. Tetrahedron, 31 (8): 929-932.

Yokota, J., D. Takuma, A. Hamada, M. Onogawa, S. Yoshioka, M. Kusunose, M. Miyamura, S. Kyotani and Y. Nishioka, 2006. Scavenging of reactive oxygen species by *Eriobotrya japonica* seed extract. Biol. Pharm. Bull., 29 (3): 467-471.

Yoshida, Y. and E. Niki, 2003. Antioxidant effects of phytosterol and its components. J. Nutr. Sci. Vitaminol. (Tokyo), 49 (4): 277-280.

Yousefipour, Z., K. Ranganna, M.A. Newaz and S.G. Milton, 2005. Mechanism of acrolein-induced vascular toxicity. J. Physiol. Pharmacol., 56 (3): 337-353.

Yuan, M., P.B. Smith, R.B. Brundrett, M. Colvin and C. Fenselau, 1991. Glutathione conjugation with phosphoramide mustard and cyclophosphamide. A mechanistic study using tandem mass spectrometry. Drug Metab. Dispos., 19 (3): 625-629.

Zhang, J., Q. Tian, S.Y. Chan, S.C. Li, S. Zhou, W. Duan and Y.Z. Zhu, 2005. Metabolism and transport of oxazaphosphorines and the clinical implications. Drug Metab. Rev., 37 (4): 611-703.

Zhang, J., Q. Tian and S.F. Zhou, 2006. Clinical pharmacology of cyclophosphamide and ifosfamide. Curr. Drug Ther., 1 (1): 55-84.

Zon, G., S.M. Ludeman, J.A. Brandt, V.L. Boyd, G. Ozkan, W. Egan and K.L. Shao, 1984. NMR spectroscopic studies of intermediary metabolites of cyclophosphamide. A comprehensive kinetic analysis of the interconversion of cis and trans-4-hydroxycyclophosphamide with aldophosphamide and the concomitant partitioning of aldophosphamide between irreversible fragmentation and reversible conjugation pathways. J. Med. Chem., 27 (4): 466-485.

Zver, S., V. Zadnik, M. Bunc, P. Rogel, P. Cernelc and M. Kozelj, 2007. Cardiac toxicity of high-dose cyclophosphamide in patients with multiple myeloma undergoing autologous hematopoietic stem cell transplantation. Int. J. Hematol., 85 (5): 408-414.