Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 3 (5): 363-372, 2008
ISSN 1816-496X

Ameliorative Effect of *Ficus hispida* Linn. Leaf Extract on Cyclophosphamide-Induced Oxidative Hepatic Injury in Rats

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

Abstract: The current study was designed to scrutinize the putative hepatoprotective potential of the methanolic leaf extract of *Ficus hispida* Linn. (FH) (400 mg kg^{-1} body weight) on cyclophosphamide (CP) elicited oxidative injury in rat liver. CP administration (150 mg kg^{-1} body weight, i.p., twice, in 2 consecutive days) caused liver injury, featuring substantial increase in serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma-glutamyl transpeptidase (GGT) and bilirubin levels. In contrast, treatment with FH significantly precluded all these alterations. CP intoxicated rats depicted a remarkable oxidative stress, as evidenced by a significant elevation in lipid peroxidation (LPO) with a concomitant decrease in the GSH activity. These changes were coupled with a marked decline in the activities of enzymic antioxidants [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and glutathione reductase (GR)] in the liver tissue of CP-administered rats. FH treated rats displayed a significant inhibition of lipid peroxidation (LPO) and augmentation of endogenous antioxidants. Taken together, these findings emphasize the hepatoprotective effect of *F. hispida* leaf extract against CP-induced oxidative liver injury. Hence, *F. hispida* might serve as a promising medicinal herb in complementary chemotherapeutic modalities.

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

INTRODUCTION

The most imperative obstacle in cancer chemotherapy is the non-specific cytotoxic action on both tumor cells and normal healthy cells (Hui *et al*., 2006). Cyclophosphamide (CP), an oxazophosphorine-alkylating agent, is extensively used as an antineoplastic drug in chemotherapeutic regimens of lymphoproliferative disorders, certain solid tumors and as an immunosuppressant in the treatment of autoimmune diseases such as nephrotic syndrome, systemic lupus erythematosus and rheumatoid arthritis (Morais *et al*., 1999). In addition, CP is of paramount importance as an immunosuppressive agent in organ and bone marrow transplant regimens (Demirer *et al*., 1996; Zincke and Woods, 1977). Use of CP as an effective chemotherapeutic agent is often restricted because of its widespread adverse side effects and toxicity (DeSouza *et al*., 2000). An ample literature implicate that elevated therapeutic dose of cyclophosphamide, could cause liver disorders (Bacon and Rosenberg, 1982; Shulman *et al*., 1980; Snover *et al*., 1989).

The prime factor for therapeutic and toxic effects of cyclophosphamide is the requirement of bioactivation by hepatic microsomal cytochrome P450 mixed functional oxidase system

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

(Lindley *et al*., 2002; Smith and Kehrer, 1991). Metabolic activation through the predominant pathway (4-hydroxylation), yields 4-hydroxycyclophosphamide (HCP) that exists in equilibrium with aldophosphamide, which degrades by β-elimination to form the DNA cross-linking agent, Phosphoramide Mustard (PM) and an equimolar amount of the toxic byproduct, acrolein (Lindley *et al*., 2002; Pass *et al*., 2005). 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 acrolein is associated with unwanted side effects (Colvin, 1999). Bioconversion of CP to these metabolites leads to the formation of high levels of Reactive Oxygen Species (ROS), which result in decreased antioxidative capacity (Stankiewicz *et al*., 2002). It is well known that excessive production of ROS could culminate in oxidative stress (Scherz-Shouval and Elazar, 2007). Mounting evidences suggest that oxidative stress play a predominant aetiological role in cyclophosphamide induced hepatotoxicity (Manda and Bhatia, 2003; Selvakumar *et al*., 2005; Stankiewicz *et al*., 2002).

Cytoprotectants like amifostine, mesna and dexrazoxane were used to manage the toxic effects of cancer chemotherapy, but these agents are not approved for wide clinical use due to lack of efficacy, gastrointestinal side effects, hypotension, hypersensitivity reactions, anxiety, urinary retention and myelosuppression (Adamson *et al*., 1995; Hensley *et al*., 1999; Reinhold-Keller *et al*., 1992). Limitations to such conventional treatment have spurred the development of new treatment modalities.

Plants are arguably poised for a comeback as sources of human health products, mainly due to their enormous propensity to synthesize complex mixtures of structurally diverse compounds, which could provide a safer and more holistic approach to disease treatment and prevention (Raskin *et al*., 2002). 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).

Ficus hispida Linn., is a shrub or moderate sized tree of the Mulberry family (Moraceae), commonly known as Peyatti (Tamil), Bhramhamedi (Telugu), Gobla (Hindi) and Dumoor (Bengali). It is found in damp localities of many parts of India and flowers and fruits practically throughout the year. Almost all parts of *Ficus hispida* are used in Indian folklore medicine for the treatment of various ailments like leucoderma, skin diseases, jaundice and as anti-poisonous. Previous reports reveal that *F. hispida* leaves contain oleanolic acid, bergapten, β-amyrin, β-sitosterol (Khan *et al*., 1991), hispidin (Huong and Trang, 2006) and phenanthroindolizidine alkaloids (Peraza-Sánchez *et al*., 2002). Interestingly, Mandal *et al*. (2000) demonstrated the protective effect of *F. hispida* leaf extract on paracetamol-induced hepatotoxicity. In this view, we conjectured that treatment with methanolic extract of *F. hispida* leaves might confer protection against cyclophosphamide-induced oxidative hepatic injury.

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. The leaves were collected and 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 (BP 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 (140±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} body weight), orally for 10 days.
Group 2 : Rats received CP dissolved in saline, intraperitoneally in a dose of 150 mg kg^{-1} body weight, twice, in 2 consecutive days (i.e., in the first 2 days of the experimental period).
Group 3 : Rats received FH extract by oral gavage (400 mg kg^{-1} body weight for 10 days).
Group 4 : Rats were administered CP as in Group 2, immediately followed by supplementation with FH extract (400 mg kg^{-1} body weight) 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. The liver tissue was immediately excised and rinsed in ice cold physiological saline and then 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

Aspartate transaminase (AST), alanine transaminase (ALT), Alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were estimated by the method of King (1965a, b, c). Gamma-glutamyl transpeptidase (GGT) was measured by the method of Szaszi (1969). Bilirubin was estimated by the Malloy and Evelyn method (1937). Protein content was estimated by the method of Lowry *et al*. (1951).

Lipid Peroxidation

Tissue lipid peroxide level was determined as MDA (Ohkawa *et al*., 1979). The absorbance was measured photometrically at 532 nm and the concentrations were expressed as µmol malonaldehyde (MDA) min^{-1} mg^{-1} protein.

Assay of Antioxidants

SOD was assayed by the method of Misra and Fridovich (1972). 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). Glutathione-S-transferase (GST) was assayed by the method of Habig *et al*. (1974). Glutathione Reductase (GR) 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 six 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, cyclophosphamide administration induced severe biochemical changes as well as oxidative damage in liver. There was a significant ($p<0.05$) rise in the levels of diagnostic marker enzymes (AST, ALT, ALP, LDH and GGT) and bilirubin in the serum of Group 2 CP intoxicated rats as compared to that of Group 1 control rats (Table 1). The administration of *Ficus hispida* leaf extract to Group 4 animals restored these enzyme as well as bilirubin levels 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.

Injection of CP induced a significant ($p<0.05$) increase in the level of lipid peroxidation (LPO), which was paralleled by significant ($p<0.05$) reduction in the level of GSH (Table 3) in the liver 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. In this study, the treatment with *F. hispida* (Group 4) significantly ($p<0.05$) counteracted the CP-induced lipid peroxidation and restored the hepatic GSH level 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 liver tissue of Group 2 CP-injected rats as compared to that of Group 1 normal control rats (Table 2). The observed reduction in the activities of GPx, GR and GST in CP-induced liver damage might be due to decreased availability of its 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.

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

Groups	AST	ALT	ALP	LDH	GGT	Bilirubin
	---------- (IU L^{-1}) ----------					(mg dL^{-1})
Group 1 (Control)	58.33±1.84	41.20±1.20	82.17±3.31	116.50±5.65	0.62±0.02	0.74±0.05
Group 2 (CP)	223.17±14.06^{a,*}	97.17±6.74^{a,*}	185.33±12.11^{a,*}	178.83±9.33^{a,*}	1.35±0.07^{a,*}	4.08±0.31^{a,*}
Group 3 (FH)	60.03±1.77^{NS}	39.77±1.58^{NS}	79.50±3.08^{NS}	122.08±5.31^{NS}	0.60±0.02^{NS}	0.81±0.04^{NS}
Group 4 (FH + CP)	67.28±4.78^{b,*}	45.77±3.25^{b,*}	90.33±6.65^{b,*}	130.67±10.75^{b,*}	0.68±0.05^{b,*}	1.42±0.07^{b,*}

Results are expressed as mean±SD for 6 rats. Comparisons are made between: ^{a}Group 1 and Group 2; ^{b}Group 3 and Group 4, *Statistically significant ($p<0.05$); NS: Non-Significant

Table 2: Effect of cyclophosphamide and *F. hispida* on the activities of liver enzymic antioxidants

Groups	SOD	CAT	GPx	GST	GR
Group 1 (Control)	11.23±0.44	144.33±7.09	116.50±5.96	12.85±0.50	2.42±0.13
Group 2 (CP)	7.98±0.74^{a,*}	105.67±8.38^{a,*}	72.33±6.28^{a,*}	6.42±0.69^{a,*}	1.20±0.13^{a,*}
Group 3 (FH)	11.65±0.81^{NS}	141.88±6.72^{NS}	114.17±7.17^{NS}	13.18±0.53^{NS}	2.56±0.17^{NS}
Group 4 (FH + CP)	10.47±0.61^{b,*}	136.00±6.03^{b,*}	109.28±6.64^{b,*}	11.78±0.59^{b,*}	2.35±0.10^{b,*}

Results are expressed as mean±SD for six rats. Units-SOD: Units/mg 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 Group 2; ^{b}: Group 3 and Group 4. *: Statistically significant ($p<0.05$); NS: Non-Significant

Table 3: Levels of GSH and LPO in the liver of the experimental animals

Groups	GSH	LPO
Group 1 (Control)	4.68±0.26	3.71±0.17
Group 2 (CP)	2.88±0.21^{a,*}	6.98±0.67^{a,*}
Group 3 (FH)	4.92±0.25^{NS}	3.95±0.11^{NS}
Group 4 (FH + CP)	4.10±0.29^{b,*}	4.61±0.32^{b,*}

Results are given as mean±SD for six rats. Units: GSH: μg mg^{-1} protein; LPO: μmoles of MDA formed min^{-1} mg^{-1} protein. Comparisons are made between: ^{a}: Group 1 and Group 2; ^{b}: Group 3 and Group 4. *: Statistically significant ($p<0.05$); NS: Non-Significant

DISCUSSION

Cyclophosphamide (CP) is a widely prescribed non-cell-cycle-specific antineoplastic drug which is known to cause toxic effects including hepatotoxicity (DeLeve, 1996). In the present study, rats intoxicated with CP displayed a substantial increase in the activities of diagnostic marker enzymes (AST, ALT, ALP, LDH and GGT) and bilirubin levels in serum, which obviously reflect a significant damage in the structural integrity of liver. Recent findings suggest that serum GGT might be a useful marker of oxidative stress (Lee *et al*., 2004). When the liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol leak into blood stream. Their estimation in the serum is a useful quantitative marker for the extent and type of hepatocellular damage (Ansari *et al*., 1991). Administration of FH attenuated the increased levels of the serum enzymes and bilirubin, produced by CP and caused a subsequent recovery towards normalization, which is fairly in line with the previous study (Mandal *et al*., 2000). This was an indication of stabilization of plasma membrane which might be attributed to the membrane stabilizing effect of the phytoconstituents like oleanolic acid and β-sitosterol, present in FH (Tang *et al*., 2005; Yokota *et al*., 2006).

Free radicals exert their cytotoxic effect by peroxidation of membrane phospholipids leading to change in permeability and loss of membrane integrity (Meerson *et al*., 1982). In our present study, intraperitoneal administration of CP resulted in an increase in lipid peroxidation (LPO) [measured by the level of malondialdehyde (MDA)] in liver. 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 ascribed to the presence of oleanolic acid, hispidin and β-sitosterol which have been reported to exhibit anti-lipid peroxidation and/or free radical scavenging properties (Balanehru and Nagarajan, 1991; Park *et al*., 2004; Yokota *et al*., 2006).

Although, the comprehensive mechanism for the hepatotoxic activity of CP remains obscure, the causal association of oxidative stress and hepatotoxicity has been supported by the observation that antioxidant therapy ameliorates hepatotoxicity (Manda and Bhatia, 2003; Selvakumar *et al*., 2005; Stankiewicz *et al*., 2002). In the present study, free radical-induced increase in LPO is accompanied by contemporaneous decline in the activities of cellular antioxidants. This may be owing to the inactivation of cellular antioxidants by lipid peroxides and ROS, which are produced due to CP

intoxication. FH administration restored these enzyme levels near to normalcy by bolstering the antioxidant defense system, which might be ascribed to the free radical scavenging/antioxidant properties of the phytochemical constituents present in FH.

GSH plays an important role in the regulation of variety of cell functions and in cell protection from oxidative injury. It is well known that depletion of GSH results in enhanced lipid peroxidation and excessive lipid peroxidation can cause increased GSH consumption, as observed in the present study. The dramatic decline in GSH level caused due to CP exposure may be attributed to the direct conjugation of CP's metabolites with free or protein bound-SH groups (Yousefipour *et al*., 2005; Yuan *et al*., 1991), thereby interfering with the antioxidant functions. Activities of the anti-peroxidative enzymes (CAT, SOD and GPx) were significantly reduced in GSH depleted condition due to pronounced oxidative stress and amassing of H_2O_2, making the cells more exposed to oxidative stress (Rajasekaran *et al*., 2002). Treatment with FH reinstated the GSH level to a near normal status. The presence of triterpenoid constituents in FH may at least in part account for this restorative effect (Liu *et al*., 1993a, 1995; Oliveira *et al*., 2005).

CP administration diminished the activities of GSH metabolizing enzymes, GST and GR. The decreased availability of GSH partly might be accountable for the reduced activity of GST and also because of its oxidative modification in its protein structure (Senthilkumar *et al*., 2006). GR is an important redox enzyme, which plays a major role in regenerating endogenous GSH from GSSG. This enzyme contains one or more sulphydryl group residues, which are essential for catalytic activity and are vulnerable to free radical mediated inactivation (Gutierrez-Correa and Stoppani, 1997). The selective reaction of acrolein with the active site sulfhydryl cysteine provides an additional evidence for the reduced GR activity (Esterbauer *et al*., 1991). FH administration augmented these antioxidant levels near to the normal status.

Interestingly, previous reports suggest that oleanolic acid has protective effect against cyclophosphamide-induced toxicities (Liu *et al*., 1995). Numerous studies corroborate the hepatoprotective effect of oleanolic acid on various models (Liu *et al*., 1993a, b; Kim *et al*., 2005; Abdel-Zaher *et al*., 2007). A recent fascinating report by Oliveira *et al*. (2005) demonstrate the hepatoprotective effect of β-amyrin through diminution of oxidative stress. Literature citations show that β-sitosterol possess antioxidant and plausible hepatoprotective properties (Nakamura *et al*., 1992; Yoshida and Niki, 2003). As cited by Malathi and Gomez (2007) phenanthroindolizidine alkaloids might have a significant role in the hepatoprotective effect. Prodigious amounts of literature data suggest that triterpenoids, flavonoids, tannins possess significant antioxidant/hepatoprotective effects (Augusti *et al*., 2005; Bai *et al*., 2007; Buniatian *et al*., 1998; Daniel *et al*., 2003). Synergistic action of these aforesaid phytoconstituents present in *F. hispida* leaf extract might be responsible for the alleviation CP induced hepatic damage.

CONCLUSION

In summary, intoxication of rats with cyclophosphamide impinged oxidative stress and liver damage, which is illuminated by dramatic elevation in the pathological parameters with a substantial drop in the antioxidant parameters. Indeed, the data presented here reveal the hepatoprotective role of *F. hispida* leaf extract, which is evidenced by the normalization of the pathological and antioxidant parameters. The present study thus pharmacologically validated the folkloric use of *F. hispida* in the treatment of liver diseases and also highlights the claim that *F. hispida* may be considered as a potentially useful candidate in the combination chemotherapy with CP to combat oxidative stress mediated liver injury. Further investigation to elucidate the protective role of *Ficus hispida* in cyclophosphamide-induced toxic manifestations is underway.

REFERENCES

Abdel-Zaher, A.O., M.M. Abdel-Rahman, M.M. Hafez and F.M. Omran, 2007. Role of nitric oxide and reduced glutathione in the protective effects of aminoguanidine, gadolinium chloride and oleanolic acid against acetaminophen-induced hepatic and renal damage. Toxicology, 234 (1-2): 124-134.

Adamson, P.C., F.M. Balis, J.E. Belasco, B. Lange, S.L. Berg, S.M. Blaney, C. Craig and D.G. Poplack, 1995. A phase I trial of amifostine (WR-2721) and melphalan in children with refractory cancer. Cancer Res., 55 (18): 4069-4072.

Ansari, R.A., S.C. Tripathi, G.K. Patnaik and B.N. Dhawan, 1991. Antihepatotoxic properties of picroliv: An active fraction from rhizomes of *Picrorhiza kurroa*. J. Ethnopharmacol., 34 (1): 61-68.

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.

Bacon, A.M. and S.A. Rosenberg, 1982. Cyclophosphamide hepatotoxicity in a patient with systemic lupus erythematosus. Ann. Int. Med., 97 (1): 62-63.

Bai, X., A. Qiu, J. Guan and Z. Shi, 2007. Antioxidant and protective effect of an oleanolic acid-enriched extract of *A. deliciosa* root on carbon tetrachloride induced rat liver injury. Asia Pac. J. Clin. Nutr., 16 (Suppl 1): 169-173.

Balanehru, S. and B. Nagarajan, 1991. Protective effect of oleanolic acid and ursolic acid against lipid peroxidation. Biochem. Int., 24 (5): 981-990.

Buniatian, N.D., V.V. Chikitkina and L.V. Iakovleva, 1998. The hepatoprotective action of ellagotannins. Eksp. Klin. Farmakol., 61 (5): 53-55.

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

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.

DeLeve, L.D., 1996. Cellular target of cyclophosphamide toxicity in the murine liver: Role of glutathione and site of metabolic activation. Hepatology, 24 (4): 830-837.

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.

DeSouza, 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.

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

Esterbauer, H., R.J. Schaur and H. Zollner, 1991. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic. Biol. Med., 11 (1): 81-128.

Gutierrez-Correa, J. and A.O. Stoppani, 1997. Inactivation of yeast glutathione reductase by Fenton systems: Effect of metal chelators, catecholamines and thiol compounds. Free Radic. Res., 27 (6): 543-555.

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.

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.

Hensley, M.L., L.M. Schuchter and C. Lindley *et al*., 1999. American society of clinical oncology clinical practice guidelines for the use of chemotherapy and radiotherapy protectants. J. Clin. Oncol., 17 (10): 3333-3355.

Hui, M.K.C., W.K.K. Wu, V.Y. Shin, W.H.L. So and C.H. Cho, 2006. Polysaccharides from the root of *Angelica sinensis* protect bone marrow and gastrointestinal tissues against the cytotoxicity of cyclophosphamide in mice. Int. J. Med. Sci., 3 (1): 1-6.

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.

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.

Kim, N.Y., M.K. Lee, M.J. Park, S.J. Kim, H.J. Park, J.W. Choi, S.H. Kim, S.Y. Cho and J.S. Lee, 2005. Momordin Ic and oleanolic acid from Kochiae Fructus reduce carbon tetrachloride-induced hepatotoxicity in rats. J. Med. Food, 8 (2): 177-183.

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

King, J., 1965b. The Hydrolases-Acid and Alkaline Phosphatases. In: Practical Clinical Enzymology, Van, D. (Ed.). Nostrand Company Limited, London, pp: 199-208.

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

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

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, D.H., R. Blomhoff and D.R. Jr. Jacobs, 2004. Is serum gamma glutamyltransferase a marker of oxidative stress? Free Radic. Res., 38 (6): 535-539.

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): 814-821.

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

Liu, Y., H. Kreppel, J. Liu, S. Choudhuri and C.D. Klaassen, 1993b. Oleanolic acid protects against cadmium hepatotoxicity by inducing metallothionein. J. Pharmacol. Exp. Ther., 266 (1): 400-406.

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.

Malathi, R. and M.P. Gomez, 2007. Hepatoprotective effect of methanolic leaves extracts of *Tylophora asthmatica* against paracetamol induced liver damage in rats. J. Pharmacol. Toxicol., 2 (8): 737-742.

Malloy, H.T. and K.A. Evelyn, 1937. The determination of bilirubin with the photometric colorimeter. J. Biol. Chem., 119: 481-490.

Manda, K. and A.L. Bhatia, 2003. Prophylactic action of melatonin against cyclophosphamide-induced oxidative stress in mice. Cell Biol. Toxicol., 19 (6): 367-372.

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.

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

Meerson, F.Z., V.E. Kagan, Y.P. Kozlov, L.M. Belkina and Y.V. Arkhipenko, 1982. The role of lipid peroxidation in pathogenesis of ischemic damage and antioxidant protection of the heart. Basic Res. Cardiol., 77 (5): 465-468.

Misra, H.P. and T. 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.

Morais, M.M., J.N. Belarmino-Filho, G.A.C. Brito and R.A. Ribeiro, 1999. Pharmacological and histopathological study of cyclophosphamide-induced hemorrhagic cystitis-comparison of the effect of dexamethasone and Mesna. Braz. J. Med. Biol. Res., 32 (10): 1211-1215.

Nakamura, H., N. Kumazawa, S. Ohta, T. Fujita, T. Iwasaki and M. Shinoda, 1992. Protective effects of the fractions extracted from the callus of Acer nikoense Maxim. on alpha-naphthylisothiocyanate induced liver injury. Yakugaku Zasshi, 112 (2): 115-123.

Noller, C.R., R.A. Smith, G.R. Harris and J.M. Walker, 1942. Saponins and sapogenins. Some color reactions of triterpenoid sapogenins. J. Am. Chem. Soc., 64: 3047.

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

Oliveira, F.A., M.H. Chaves, F.R.C. Almeida, R.C.P. Jr. Lima, R.M. Silva, J.L. Maiaa, 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.

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.

Peraza-Sánchez, S.R., H. 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*. Plant. Med., 68 (2): 186-188.

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.

Raskin, I., D.M. Ribnicky, S. Komarnytsky and N. Ilic *et al*., 2002. Plants and human health in the twenty-first century. Trends Biotechnol., 20 (12): 522-531.

Reinhold-Keller, E., J. Mohr, E. Christophers, K. Nordmann and W.L. Gross, 1992. Mesna side effects which imitate vasculitis. Clin. Invest., 70 (8): 698-704.

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.

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

Selvakumar, E., C. Prahalathan, Y. Mythili and P. Varalakshmi, 2005. Mitigation of oxidative stress in cyclophosphamide-challenged hepatic tissue by DL-alpha-lipoic acid. Mol. Cell. Biochem., 272 (1-2): 179-185.

Senthilkumar, S., K.K. Ebenezar, V. Sathish, S. Yogeeta and T. Devaki, 2006. Modulation of the tissue defense system by squalene in cyclophosphamide induced toxicity in rats. Arch. Med. Sci., 2 (2): 94-100.

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.

Shulman, H.M., G.B. McDonald, D. Matthews, K.C. Doney, K.J. Kopecky, J.M. Gauvreau and E.D. Thomas, 1980. An analysis of hepatic venocclusive disease and centrilobular hepatic degeneration following bone marrow transplantation. Gastroenterology, 79 (6): 1178-1191.

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

Smith, R.D. and J.P. Kehrer, 1991. Cooxidation of Cyclophosphamide as an alternative pathway for its bioactivation and lung toxicity. Cancer Res., 51 (2): 542-548.

Snover, D.C., S. Weisdorf, J. Bloomer, P. McGlave and D. Weisdorf, 1989. Nodular regenerative hyperplasia of the liver following bone marrow transplantation. Hepatology, 9 (3): 443-448.

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.

Stankiewicz, A., E. Skrzydlewska and M. Makiela, 2002. Effects of amifostine on liver oxidative stress caused by cyclophosphamide administration to rats. Drug Metabol. Drug Interact., 19 (2): 67-82.

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

Szaszi, G., 1969. A kinetic photometric method for serum gamma-glutamyl transpeptidase. Clin. Chem., 15 (2): 124-126.

Tang, X.H., J. Gao, F. Fang, J. Chen, L.Z. Xu, X.N. Zhao and Q. Xu, 2005. Hepatoprotection of oleanolic acid is related to its inhibition on mitochondrial permeability transition. Am. J. Chin. Med., 33 (4): 627-637.

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.

Zincke, H. and J.E. Woods, 1977. Donor pretreatment in cadaver renal transplantation. Surg. Gynecol. Obstet., 145 (2): 183-188.