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

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**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; Itsesu 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 phosphoramid mustard (PM) and acrolein (Murgu 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...
possess the ability to minimize the noxious effects induced by chemotherapy to normal cells with or without 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 Iha, 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 been studied extensively, but the isolation of phananthrindolizidine 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, bengapten, β-amyrin and β-sitosterol (Huang 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; Khushbakhtova 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 (Lodoxan®) 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 (Liebmann, 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 Tamil Nadu 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 hydrazoform 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

<table>
<thead>
<tr>
<th>Groups</th>
<th>CPK (IU L⁻¹)</th>
<th>LDH (IU L⁻¹)</th>
<th>AST (IU L⁻¹)</th>
<th>ALT (IU L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Control)</td>
<td>128.1 ± 6.55</td>
<td>265.9 ± 11.95</td>
<td>83.8 ± 6.07</td>
<td>548.7 ± 2.9</td>
</tr>
<tr>
<td>2 (CP)</td>
<td>265.8 ± 22.9**</td>
<td>428.1 ± 37.0**</td>
<td>276.7 ± 25.3**</td>
<td>180.6 ± 15.3**</td>
</tr>
<tr>
<td>3 (FH)</td>
<td>125.7 ± 2.93</td>
<td>269.7 ± 12.2**</td>
<td>83.5 ± 2.35**</td>
<td>53.1 ± 1.47**</td>
</tr>
<tr>
<td>4 (FH + CP)</td>
<td>138.1 ± 8.42</td>
<td>278.0 ± 14.2**</td>
<td>93.3 ± 8.50**</td>
<td>60.7 ± 1.56**</td>
</tr>
</tbody>
</table>

Results are expressed as mean±SD for 6 rats. Comparisons are made between: 1Group 1 and 2; 2Group 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

<table>
<thead>
<tr>
<th>Groups</th>
<th>CPK (IU mg⁻¹ protein)</th>
<th>LDH (IU mg⁻¹ protein)</th>
<th>AST (IU mg⁻¹ protein)</th>
<th>ALT (IU mg⁻¹ protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Control)</td>
<td>22.47 ± 1.12</td>
<td>33.60 ± 1.46</td>
<td>7.09 ± 0.31</td>
<td>6.03 ± 0.19</td>
</tr>
<tr>
<td>2 (CP)</td>
<td>7.25 ± 0.49**</td>
<td>14.83 ± 1.36**</td>
<td>3.18 ± 0.58*</td>
<td>2.76 ± 0.11**</td>
</tr>
<tr>
<td>3 (FH)</td>
<td>23.25 ± 0.89**</td>
<td>35.05 ± 1.33**</td>
<td>7.45 ± 0.35**</td>
<td>5.89 ± 0.41**</td>
</tr>
<tr>
<td>4 (FH + CP)</td>
<td>17.91 ± 0.94**</td>
<td>28.57 ± 1.7**</td>
<td>7.03 ± 0.67**</td>
<td>4.94 ± 0.17**</td>
</tr>
</tbody>
</table>

Results are expressed as mean±SD for 6 rats. Comparisons are made between: 1Group 1 and 2; 2Group 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

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (Units mg⁻¹ protein)</th>
<th>CAT (μmol H₂O₂ consumed min⁻¹ mg⁻¹ protein)</th>
<th>GPx (μmol min⁻¹ mg⁻¹ protein)</th>
<th>GST (μmol min⁻¹ mg⁻¹ protein)</th>
<th>GR (μmol min⁻¹ mg⁻¹ protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Control)</td>
<td>5.15 ± 0.11</td>
<td>32.06 ± 0.41</td>
<td>1.86 ± 0.3</td>
<td>0.82 ± 0.04</td>
<td>1.49 ± 0.06</td>
</tr>
<tr>
<td>2 (CP)</td>
<td>2.58 ± 0.08**</td>
<td>18.69 ± 0.42**</td>
<td>0.83 ± 0.04**</td>
<td>0.43 ± 0.02**</td>
<td>0.72 ± 0.03**</td>
</tr>
<tr>
<td>3 (FH)</td>
<td>5.07 ± 0.04**</td>
<td>33.27 ± 0.21**</td>
<td>1.91 ± 0.06**</td>
<td>0.83 ± 0.05**</td>
<td>1.54 ± 0.03**</td>
</tr>
<tr>
<td>4 (FH + CP)</td>
<td>4.88 ± 0.06**</td>
<td>29.19 ± 0.55**</td>
<td>1.71 ± 0.07**</td>
<td>0.75 ± 0.02**</td>
<td>1.47 ± 0.03**</td>
</tr>
</tbody>
</table>

Results are expressed as mean±SD for 6 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: μmol H₂O₂ consumed min⁻¹ mg⁻¹ protein. GPx: μmol GSH oxidized min⁻¹ mg⁻¹ protein. GST: μmol CDNB (1-chloro-2,4-dinitrobenzene) conjugated min⁻¹ mg⁻¹ protein. GR: μmol NADPH oxidized min⁻¹ mg⁻¹ protein. Comparisons are made between: 1Group 1 and 2; 2Group 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.

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 (Guttoo 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-Shabarah 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 oleoacidic 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 (H₂O₂), 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 oleoacidic 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 (H₂O₂) while GPx and CAT by an excess of superoxide radical (Pigeot et al., 1990). In fact, the heart has a greater susceptibility to oxidative stress than other tissues due to its inherent decreased detoxifying antioxidants (Doroshov 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 H₂O₂ 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 H₂O₂, more H₂O₂ 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 sulphydryl (–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 H₂O₂ 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 oleic acid has protective effect against cyclophosphamide-induced toxicities (Liu, 1995). Recent evidence suggests that oleic 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


