Chapter 2

Hepatoprotective Activity of Aqueous Extracts of *Phyllanthus fraternus* and *Aegle marmelos* against Cyclophosphamide-Induced Toxicity

5.1. Introduction

The liver is an important organ responsible for metabolism, bile secretion, elimination of many substances, blood detoxification, synthesis and regulation of essential hormones. It is a major metabolic organ affected by various chemicals and toxins daily. Liver diseases have become a worldwide problem and are associated with significant morbidity and mortality. The principal causative factors for liver diseases in developed countries are excessive alcohol consumption and virus-induced chronic liver diseases, while in developing countries the most frequent causes are environmental toxins, parasitic disease, hepatitis B and C viruses and hepatotoxic drugs such as certain antibiotics, chemotherapeutic agents, high doses of paracetamol, carbon tetrachloride, thioacetamide etc. Chronic liver cirrhosis and drug induced liver injury accounting the ninth leading cause of death in western and developing countries. Drug-induced liver injury is a major health problem that challenges not only health care professionals, but also the pharmaceutical industry and drug regulatory agencies (Saleem *et al.*, 2008).

CPA is an alkylating agent, the most commonly used anticancer and immunosuppressant drug. It is used for the treatment of chronic and acute leukemias, multiple myeloma, lymphomas, rheumatic arthritis and in preparation for bone marrow transplantation (Goldberg *et al.*, 1986; Dollery, 1999). Phosphoramidate mustard and acrolein are two active metabolites of CPA produced by liver
microsomal enzymes (Ludeman, 1999). Cyclophosphamide’s antineoplastic effects are associated with phosphoramidemustard, while acrolein is linked with its toxic side effects (Kern and Kehrer, 2002). Liver disorders were observed in the increased therapeutic dose of CPA (Atkinson et al., 1987; Snover et al., 1989). Therapeutic dose of CPA causes liver disorders and nephrotoxicity which leads to gonadal toxicity, as a side effect of the drug (Senthilkumar et al., 2006). To avoid these toxic side effects, some antioxidant agents should be used to detoxify the acrolein. Some cytoprotective drugs like amifostine, a thiol antioxidant had been tried but not found effective due to its side effects such as hypocalcaemia, anxiety and hypotension (Shaw and Blekley, 2000). Therefore, there is a need for novel agents, which may protect normal tissue from chemotherapy induced toxicity with the absence of tumor protection and tumor growth stimulation properties. In the absence of reliable hepatoprotective drugs, a large number of herbal preparations are widely used for the treatment of liver disorders (Chatterjee, 2000).

Plants have played a significant role in maintaining human health and improving the quality of human life from thousands of years. They are good source of foods, medicines, seasonings, beverages, cosmetics and dyes. Herbal medicines are popular in recent time in the treatment of human health complications due to its safe and no side effects. Currently, demand of herbal plants has increased all over the world for production of a large number of Ayurvedic formulations. Today, we are witnessing a great deal of public interest in the use of herbal remedies. Furthermore many western drugs had their origin in plant extract. There are many herbs, which are predominantly used to treat cardiovascular problems, liver disorders, central nervous system, digestive and metabolic disorders. Due to significant therapeutic value, they may be used as drug or supplement in the treatment or management of various diseases. Extracts as well as compounds isolated from herbal plants have wide spectrum of biological activities. Ethnopharmacological studies developed the confidence of people in the world in the treatment of traditional system of medicines.
In this study, we have investigated the ability of aqueous extracts of *P. fraternus* (AEPF) and *A. marmelos* (AEAM) to protect the liver against CPA-induced hepatocellular damage and oxidative stress in mice.

5.2. Materials and Methods

5.2.1. Animals and Treatment: Adult (age 12-15 weeks) male laboratory mice of Parke’s strain weighing 30 ± 3 gm were used in the investigation. Mice were allocated into 12 groups (group I-XII), each group comprising of six animals. In a group, all animals had more or less identical body weight (bw). Mice in each group were housed separately in polypropylene cage (430 mm X 270 mm X 150 mm) and the treatment was done as shown below:

- **Group I**  Control mice received distilled water (intraperitoneally) once in a week for 5 weeks.
- **Group II**  AEPF (400 mg/kg bw, orally) once in a week for 5 weeks.
- **Group III**  AEAM (600 mg/kg bw, orally) once in a week for 5 weeks.
- **Group IV**  Silymarin 100 mg/kg bw, for 5 weeks (once in a week) orally.
- **Group V**  Received CPA 200 mg/kg bw, for 5 weeks (once in a week) by intraperitoneal injection.
- **Group VI-VIII**  Received CPA (200 mg/kg bw, intraperitoneally) and AEPF 200, 300 and 400 mg/kg bw, orally for 5 weeks (once in a week).
- **Group IX-XI**  Received CPA (200 mg/kg bw, intraperitoneally) and AEAM 400, 500 and 600 mg/kg bw, orally for 5 weeks (once in a week).
- **Group XII**  Received CPA (200 mg/kg bw, intraperitoneally) and silymarin (well known standard hepatoprotective drug) 100 mg/kg bw, orally for 5 weeks (once in a week).

After 5 weeks all the animals were sacrificed by dislocation of cervical vertebrae. The liver was excised, blotted free of blood and fixed in Bouin’s solution for histopathological analysis by H & E staining. Blood was collected and serum was separated for analysis of biochemical parameters. Details of the procedure of the
biochemical, haematology and histopathology estimation are described in materials and methods.

5.2.2. Statistical Analysis: The results were expressed as Mean ± SE (SEM), analyzed through one-way ANOVA, followed by the post hoc Dunnett’s test for comparison of various treatments using the SPSS 16.0. Differences were considered statistically significant at p<0.05.

5.3. Results

5.3.1. Effect of AEPF and AEAM on Serum Transaminases (SGOT and SGPT) Activity against CPA-Induced Toxicity in Mice: The effects of CPA with AEPF and AEAM on concentration of serum SGOT and SGPT levels are shown in Figure 5.1. CPA intoxicated (group V) serum SGOT, SGPT levels were significantly increased (p<0.001), as compared to the control (group I). It indicates the hepatotoxicity in mice caused by CPA. However, the administration of AEPF and AEAM at different doses with CPA significantly reduced the elevated levels of serum SGOT and SGPT in a dose-dependent manner, respectively (group VI to XI). At low doses of AEPF (200 and 300 mg/kg) and AEAM (400 and 500 mg/kg), the effect was only marginal, whereas at the higher doses of AEPF (400 mg/kg) and AEAM (600 mg/kg) effectively prevented the CPA-induced liver damage (Figure 5.1). Mice treated with silymarin or a higher dose of extracts alone as well as co-treatment of silymarin and CPA subjected to the biochemical parameter analysis revealed that the values were near to the control group.

5.3.2. Effect of AEPF and AEAM on Serum Phosphatases (ALP and ACP) Activity against CPA-Induced Toxicity in Mice: The effects of CPA with AEPF and AEAM on concentrations of serum ALP and ACP levels are shown in Figure 5.2. CPA intoxicated group (V) serum ALP (p<0.001) and ACP (p<0.001) levels were increased significantly, as compared to the control (group I). It indicates
the hepatotoxicity in mice caused by CPA. However, the administration of AEPF and AEAM at different doses with CPA effectively reduced the elevated levels of serum ALP and ACP in a dose-dependent manner (group VI to XI). At low doses of AEPF (200 and 300 mg/kg) and AEAM (400 and 500 mg/kg), the effect was only marginal, whereas at the higher doses of AEPF (400 mg/kg) and AEAM (600 mg/kg) effectively prevented the CPA-induced liver damage (Figure 5.2). Mice treated with silymarin or a higher dose of extracts alone as well as co-treatment of silymarin and CPA subjected to the biochemical parameter analysis, revealed that the values were near to the control group.

5.3.3. Effect of AEPF and AEAM on Serum LDH and Cholesterol Levels against CPA-Induced Toxicity in Mice: The effects of CPA with AEPF and AEAM on concentrations of serum LDH and cholesterol levels are shown in Figure 5.3. CPA intoxicated group (V) serum LDH and cholesterol \( (p<0.001) \) levels were increased significantly, as compared to the control (group I). It indicates the hepatotoxicity in mice caused by CPA. However, the administration of AEPF and AEAM at different doses with CPA effectively reduced the elevated levels of serum LDH and cholesterol in a dose-dependent manner (group VI to XI). At low doses of AEPF (200 and 300 mg/kg) and AEAM (400 and 500 mg/kg), the effect was only marginal, whereas at the higher doses of AEPF (400 mg/kg) and AEAM (600 mg/kg) effectively prevented the CPA-induced liver damage (Figure 5.3). Mice treated with silymarin or a higher dose of extracts alone as well as co-treatment of silymarin and CPA subjected to the biochemical parameter analysis, revealed that the values were near to the control group.

5.3.4. Effect of AEPF and AEAM on Serum Total Bilirubin and Albumin Levels against CPA-Induced Toxicity in Mice: The effects of CPA with AEPF and AEAM on concentrations of serum bilirubin and albumin levels are shown in Figure 5.4. Level of total bilirubin significantly increased \( (p<0.001) \) in
group II, whereas level of albumin significantly decreased ($p<0.001$) in group II as compared to control. The animals treated with AEPF and AEAM at different dose exhibited a decrease in total bilirubin along with an increase in albumin significantly ($p<0.001$). It is evident from Figure 5.4, that the AEPF and AEAM reversed the toxicity of CPA in a dose dependent manner.

5.3.5. Effect of AEPF and AEAM on Lipid Peroxidation (LPO) in Liver against CPA-Induced Toxicity in Mice: Malondialdehyde (MDA) level is widely used as a marker of free radical mediated lipid peroxidation injury of the liver. The results of antioxidant activity of aqueous extracts on CPA-intoxicated mice are shown in Figure 5.5, which clearly revealed that a striking increase in the MDA levels in CPA (group II) intoxicated mice as compared to control group. Co-treatment with different doses of AEPF and AEAM exhibited significant reductions in CPA-induced MDA elevations. At the highest doses of extracts, significantly ($p<0.001$) inhibited the formation of MDA in the liver during CPA damage. At low doses of AEPF (200 and 300 mg/kg) and AEAM (400 and 500 mg/kg), the effect was only marginal, whereas at the higher doses of AEPF (400 mg/kg) and AEAM (600 mg/kg) effectively prevented the CPA-induced liver damage. Mice treated individually with AEPF, AEAM or silymarin showed a near normal level of MDA comparable to the control group.

5.3.6. Effect of AEPF and AEAM on SOD Activity in Liver against CPA-Induced Toxicity in Mice: The activity of antioxidant enzyme SOD was significantly decreased ($p<0.001$) in CPA-treated groups as compared to the control group (Figure 5.6). CPA + silymarin and different doses of AEPF and AEAM received groups significantly ($p<0.001$) increased the levels of SOD. The outcome suggests that silymarin and all the doses of AEPF and AEAM increase the level of SOD, but at higher doses of AEPF (400 mg/kg) and AEAM (600 mg/kg) was more
effective in increasing the level of SOD in CPA-treated mice as compared with other doses of extracts.

5.3.7. Effect of AEPF and AEAM on CAT in Liver against CPA-Induced Toxicity in Mice: Activity of CAT was significantly decreased \((p<0.001)\) in CPA-treated groups when compared with the control group. CPA + silymarin and different doses of AEPF and AEAM received mice show significant \((p<0.001)\) increase in CAT level. The data suggest that high doses of AEPF (400 mg/kg) and AEAM (600 mg/kg) was more effective to increase the activity of CAT in CPA-treated mice (Figure 5.6)

5.3.8. Effect of AEPF and AEAM on Haematological Parameters against CPA-Induced Toxicity in Mice: The effect of AEPF and AEAM on RBC and WBC of CPA treated animals is given in Figure 5.7. Initially, there was a significant decrease \((p<0.001)\) in the RBC and WBC of CPA treated mice, but later RBC and WBC was found to be significantly higher \((p<0.001)\) in CPA + extracts treated group. The results indicate that the administration of extracts could stimulate the haemopoietic system.

5.3.9. Histopathological Study of Liver: Histopathological photomicrographs of liver sections from various treatment groups are shown in Figure 5.8. Microscopical examination of liver sections of the control group, showed normal architecture of the liver with distinct hepatic cells (group I). However, treatment of extracts and silymarin alone also showed normal histopathology of liver (group II to IV). The liver sections of CPA intoxicated group showed picnotic nuclei and inflammation in the centrilobular region (group V). The mice treated with AEPF and AEAM at different doses, showed recovery from CPA-induced liver damage as evident from normal hepatocytes and with higher dose showed significant attenuation of inflammation and normal cellular architecture of liver was preserved, indicating a marked protective activity similar to that observed in silymarin treated mice liver
sections and the effect was found to be dose dependent (group VI to IX). The liver sections of CPA + silymarin treated mice also showed a normal hepatic architecture with normal hepatocytes (group XII).

5.4. Discussion

CPA is a well-known chemotherapeutic drug that is used in cancer treatment. The side effect of this drug is fatal hepatic damages in humans and experimental animals (Snover 1989; Dollery, 1999). Thus, it may be considered as a hepatotoxic agent (Shokrzadeh et al., 2014). An acute toxicity study of the AEPF and AEAM revealed the non-toxic nature of the extracts. There was no lethality found in the groups, which received different doses of the extracts until the end of the experimental period.

Hepatic cells participate in a variety of metabolic activities and contain several enzymes. Serum transaminases (SGOT and SGPT) are the universally important markers for hepatic tissue injury. Serum transaminases are the cytoplasmic enzymes involved in amino acid metabolisms. Liver marker enzymes are localized in the cytosol of hepatic cells and thus are extruded into the serum when cells are damaged or necrotic. In healthy subjects, serum transaminases levels are low. However, when cells are damaged, transaminases may leak into the blood stream and the level of serum transaminases increases. Therefore, determination of serum transaminases has great clinical and diagnostic significance (Rceci et al., 2006). Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Drotman and Lawhorn, 1978). In the present study, CPA-induced hepatotoxicity is manifested by increased levels of serum transaminases (SGOT and SGPT). This indicated the presence of necrotic cells that resulted leakage of these enzymes to serum. Our results are parallel to that of the previously reported by Shanmugarajan et al. (2008), who reported that, CPA administration induced significant increase in serum transaminases. Combined therapy with AEPF and AEAM prevented CPA-induced toxic consequences and restored the serum
transaminases as in control. The reduction in levels of serum enzymes by extracts is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CPA (Habibi et al., 2014).

Serum phosphatases (ALP and ACP) are involved in a variety of metabolic activities such as permeability, growth and cell differentiation, protein synthesis, absorption and transport of nutrients, gonadal maturity, steroidogenesis etc. In the result, significantly increase in serum phosphatases in CPA-intoxicated mice were observed (Manda and Bhatia, 2003; Selvakumar et al., 2005; Senthilkumar et al., 2006; Shanmugarajan et al., 2008). Thus increase in the serum phosphatase in CPA treated mice indicates hepatic cells damage. Elevated levels of serum ALP may be due to increased synthesis in presence of increasing biliary pressure (Muriel et al., 1992). Administration of different doses of AEPF and AEAM significantly normalized the above parameters as compared to CPA intoxicated mice. The above changes may be considered as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchyma cells. Thus the decreased level of serum phosphatases suggests the hepatoprotective potential of AEPF and AEAM against CPA.

LDH catalyses the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD⁺ (Pathak and Vinayak, 2005). According to the results obtained, after CPA administration, there is an increase in LDH levels in serum and liver showing increased rate of glycolysis with excess production of pyruvate than its utilization by Krebs cycle leading to lactate synthesis. An increase serum LDH in CPA-induced intoxicated mice substantiated the hepatic damage (Shanmugarajan et al., 2008). The mice treated with different doses of AEPF and AEAM showed a significant decrease in LDH in dose dependent manner, which indicate the restoration of the LDH levels near to normal values.

Bilirubin is one of the most useful clinical parameter to diagnose severity of hepatic necrosis. It is the breakdown product of haem in red blood cells in the liver. High
levels will cause jaundice and are indicative of damage to the liver and bile duct (Rajesh et al., 2005; Dubey and Mehta, 2014). Free bilirubin is insoluble in water and must be bound to albumin to facilitate transport to the liver. This indirect or unconjugated bilirubin fraction therefore does not enter urine. Once taken up by hepatocytes, bilirubin is conjugated by Uridine 5'-diphospho-glucuronosyltransferase (UDPGT) enzymes, with the glucuronide conjugates being excreted in bile. Intestinal bacteria metabolize direct bilirubin to urobilinogen, which is mainly excreted in faeces. A minor portion undergoes enterohepatic circulation, with small quantities excreted in urine. Total serum bilirubin concentrations indicate the functional transport capacity of the liver. Mild to moderate serum unconjugated hyperbilirubinemia is generally associated with haemolysis (Friedman et al., 1996) and higher concentration of bilirubin in serum is associated with abnormalities of liver functions, which indicate a hepatic etiology. If hepatic parenchymal damage is severe, less bilirubin will be excreted and hyperbilirubinemia is observed that reflect pathophysiology of liver damage. Increase in total serum bilirubin concentration after CPA administration might be attributed to the failure of normal uptake, conjugation and excreted by the damaged hepatic parenchyma. AEPF, AEAM and silymarin showed decreased levels of serum bilirubin, which suggests that it may be used as protectant for jaundice.

Cholesterol is an essential structural component of mammalian cell membrane and it is required to establish proper membrane permeability and fluidity. In the present study, the liver lipid profile such as cholesterol was significantly elevated in serum and this indicated deterioration in hepatic function due to the damage caused by CPA administration. CPA-induced elevation in cholesterol levels could be due to increase in biosynthesis and decrease in its utilization. CPA generates free radicals, which may cause cellular cholesterol accumulation, (a) by increasing cholesterol biosynthesis and its esterification, (b) by decreasing cholesterol ester hydrolysis and (c) by reducing cholesterol efflux (Gesquiere et al., 1999). The conversion of cholesterol to bile acids is quantitatively the most important mechanism for degradation of cholesterol. The
elevated level of cholesterol was significantly reduced in mice treated with AEPF and AEAM as with silymarin.

Albumin is an essential protein for maintaining the osmotic pressure needed for proper distribution of body fluids between blood vessels and body tissues. Serum albumin, the major plasma protein synthesized in the human liver, is a clinically useful marker of hepatic synthetic function (Friedman et al., 1996). The level of albumin in serum with CPA administered mice was significantly decreased. Furthermore, the hepatoprotective effect of AEPF and AEAM appeared to be as beneficial as silymarin.

In normal conditions, organs possess a powerful antioxidant defence system, such as SOD and CAT (Fuchs and Hewitt, 2011). There are a number of evidence that oxidative stress, produced by ROS, plays a vital role in the pathogenesis of CPA-induced hepatic damage (Manda and Bhatia, 2003; Selvakumar et al., 2005; Shanmugarajan et al., 2008). The removal and neutralization of these noxious toxic metabolites are considered to be the vital initial steps in the prevention of CPA-related liver diseases.

In our study, we found that CPA administration significantly increased the hepatic MDA levels and markedly decreased SOD and CAT levels. Similar findings have been reported by many investigators (Manda and Bhatia, 2003; Selvakumar et al., 2005; Shanmugarajan et al., 2008).

Lipid peroxidation (LPO) is one of the most important indicators of oxidative stress. Unsaturated fatty acids present in cellular membranes are a common target for ROS. Lipid components of the cell are especially susceptible to reactions with free radicals, resulting in LPO. Lipid peroxidation refers to the oxidative degradation of lipids. It is the action of abstraction of a hydrogen atom from the side chain of polyunsaturated fatty acids in the membrane. The present data revealed that CPA administration produced a marked oxidative impact, as evidenced by the significant increase in
MDA levels. From the other hand, treatment with, the different doses of AEPF and AEAM afforded better protection through decreased production of free radicals derivatives, as is evident from the decreased levels of MDA in a dose dependent manner.

Superoxide is believed to be the cause of other ROS formations such as hydrogen peroxide and hydroxyl radicals. Therefore, superoxide scavenging capacity in the body is the first line of defence against oxidative stress. Superoxide anions have been suggested as a major cause of CPA toxicity (Reeci et al., 2006). The decreased activity of SOD in the present study might be the reason of oxidative damage in CPA treated animals. Catalase is a common enzyme found in nearly all living organisms exposed to oxygen. It catalyses the hydrogen peroxide (H₂O₂) to water and oxygen. It is a very important enzyme in protecting the cells from oxidative damage by ROS (Foyer and Shigeoka, 2011). In the present investigation, it has been found that CPA-induced toxicity by damaging the antioxidant defence system of organs such as SOD and CAT in liver. However, treatment of mice, with AEPF and AEAM showed a strong protective potential against oxidative stress caused by CPA.

The results of the present study showed that co-administration of varied doses of AEPF and AEAM diminished CPA-induced oxidative stress by increasing antioxidant status. These results support the hypothesis that oxidative damage is neutralized when antioxidants such Ficus hispida, squalene and DL-α-lipoic acid are administered before or after the induction of oxidative stress (Selvakumar et al., 2005; Senthilkumar et al., 2006; Shanmugarajan et al., 2008).

The present study indicates that AEPF and AEAM significantly enhanced the RBC and WBC count as compared to the CPA intoxicated mice. These observations assume great significance, as anaemia is a common complication in cancer. The situation aggravates further during chemotherapy, since a majority of antineoplastic agents exerts suppressive effects on erythropoiesis and thereby limiting the use of drugs (Sreelatha et al., 2012).
The hepatoprotective effect of AEPF and AEAM was confirmed by histopathological examination of the liver of controlled and treated animals. In the present study, the histological architecture of CPA treated liver section showed the degeneration of hepatocytes. Whereas the mice treated with AEPF and AEAM at different doses showed recovery of liver from CPA-induced liver damage in a dose dependent manner. The best protection in architecture of liver was found at higher doses of AEPF and AEAM, which was found to be parallel to control and silymarin treated group. Hence, the histopathological examinations of AEPF and AEAM treated groups of mice showing hepatoprotective effects and this is supported by biochemical studies.

The hepatoprotective activity of extracts has been compared against CPA-induced toxicity in liver. Although both extracts were found with marked level of protection but AEAM had shown better efficacy than AEPF, as depicted by its level of percentage protection (Table 5.1).

Phytochemical screening of the P. fratnus and A. marmelos extracts revealed the presence of alkaloids, tannins, saponin, flavonoids and phenols, which are able to scavenge free radicals such as superoxide or lipid peroxides (Gutteridge and Halliwell, 2000). In the present study, different chemical constituents have been identified from extracts by GC-MS analysis (Table 4.2 and 4.3). The presence of various bioactive compounds justifies the use of plants for treating various ailments by traditional practitioners.

Recent work highlights the potential role of phytochemical components, including the phenols, flavonoids, tannins, saponine alkaloids etc. In the last decade, epidemiological studies had suggested the importance of plant polyphenols against degenerative diseases (Wanasundara and Shahidi, 1998). The over abundance of phenolic compounds from several plant extracts have been reported to possess strong antioxidant activities (Anish et al., 2013). The phenolic groups may accept an electron to form relatively stable phenoxy radicals, thereby disrupting chain
oxidation reactions in cellular components (Manian et al., 2008). There are increasing evidences that as antioxidants, phenols may protect cell constituents against oxidative damage and, therefore, limit the risk of drug induced with oxidative stress (Wanasundara and Shahidi, 1998; Maniana et al., 2008). The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh et al., 2007). They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Han et al., 2007).

Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds (Brown and Rice-Evans, 1998; Krings and Berger, 2001). Natural antioxidants mainly derived from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc. (Ali et al., 2008).

The biological, pharmacological and medicinal properties of the flavonoids have been extensively reviewed (Cody et al., 1986, 1988; Das, 1990). Flavonoids have multiple biological activities including vasodilatory, anticarcinogenic, antiinflammatory, antibacterial, immune-stimulating, antiallergic, antiviral and estrogenic effects. The chemistry of the flavonoids is predictive of their free radical scavenging activity (Jovanovic et al., 1992; Wardman, 1989). Their antioxidant activity is also reported as scavengers of superoxide radical (Yuting, et al., 1990; Zhou and Zheng, 1991; Cotelle, et al., 1992; Hanasaki et al., 1994). Therefore, the flavonoids were found to have higher radical scavenging activity and reducing power of free radicals (Rice-Evans, 2001).

Tannins have been considered as “health-promoting” components in plant derived foods and beverages e.g. some red wines, teas and unripe fruits. Tannins have been reported to possess anticarcinogenic, antimutagenic potentials, antimicrobial, antioxidant and antiradical activities (Amarowicz, 2007). Saponins are attributed with cardio-protective, immunomodulatory, antifatigue, hepato-protective, physiological
and pharmacological effects (Attele et al., 1999). The protective role of saponin has been reported to improve the defense mechanism under several stress conditions (Attele et al., 1999; Chen et al., 2003; Kim et al., 2005). It has been recognized that alkaloids show antioxidant activity (Kumpulainen and Salonen, 1999). Mechanisms of action of alkaloids are through inhibition of peroxidation (Cook and Samman, 1996; Kessler et al., 2003). According to the study, the high contents of these phytochemicals in A. marmelos may explain its high lipid preoxidation inhibition activity. The results obtained in this study thus suggest that the identified phytochemical compounds may be bioactive constituents and protect the drug induced hepatotoxicity in a dose dependent manner. The higher doses were found to be the best for protection against CPA-induced toxicity in mice in the present study. Therefore, this study provides an experimental evidence for the protective and beneficial role of AEPF and AEAM against CPA intoxication.

5.5. Conclusion

The experimental results suggest that the plant P. fraternus and A. marmelos are hepatoprotectant. Different active components present in AEPF and AEAM, may be responsible for hepatoprotectivity. The present study suggested that both extracts have a preventive and curative effect in CPA-induced hepatotoxicity in mice. From the above study, we may conclude that these plants have medicinal properties. However, further investigations and analysis are required in order to establish the active compounds which are responsible for the hepatoprotectivity. Thus, it may be hypothesized that AEPF and AEAM protect the liver tissues by scavenging the toxic metabolites, which is evidenced by the normalization of the clinical chemistry.
**Figure 5.1:** Protective effect of aqueous extracts of *P. fraternus* and *A. marmelos* in serum transaminases against CPA-induced hepatotoxicity. ‘a’ indicates the significant difference between the control and CPA treated groups, ‘b’ indicates the significant difference between the CPA treated and AEPF and AEAM treated groups. Each bar represents the Mean ±SE, n=6.

CPA + P : Cyclophosphamide + Aqueous extract of *P. fraternus*

CPA + A : Cyclophosphamide + Aqueous extract of *A. marmelos*

CPA + SIL: Cyclophosphamide + Silymarin
Figure 5.2: Protective effect of aqueous extracts of *P. fraternus* and *A. marmelos* in serum phosphatases against CPA-induced hepatotoxicity. ‘a’ indicates the significant difference between the control and CPA treated groups, ‘b’ indicates the significant difference between the CPA treated and AEPF and AEAM treated groups. Each bar represents the Mean ±SE, n=6.

CPA + P : Cyclophosphamide + Aqueous extract of *P. fraternus*

CPA + A : Cyclophosphamide + Aqueous extract of *A. marmelos*

CPA + SIL: Cyclophosphamide + Silymarin
Figure 5.3: Protective effect of aqueous extracts of *P. fraternus* and *A. marmelos* in serum LDH and cholesterol against CPA-induced hepatotoxicity. ‘a’ indicates the significant difference between the control and CPA treated groups, ‘b’ indicates the significant difference between the CPA treated and AEPF and AEAM treated groups. Each bar represents the Mean ±SE, n=6.

CPA + P: Cyclophosphamide + Aqueous extract of *P. fraternus*

CPA + A: Cyclophosphamide + Aqueous extract of *A. marmelos*

CPA + SIL: Cyclophosphamide + Silymarin
**Figure 5.4:** Protective effect of aqueous extracts of *P. fraternus* and *A. marmelos* in serum total bilirubin and albumin against CPA-induced hepatotoxicity. ‘a’ indicates the significant difference between the control and CPA treated groups, ‘b’ indicates the significant difference between the CPA treated and AEPF and AEAM treated groups. Each bar represents the Mean ±SE, n=6.

- CPA + P: Cyclophosphamide + Aqueous extract of *P. fraternus*
- CPA + A: Cyclophosphamide + Aqueous extract of *A. marmelos*
- CPA + SIL: Cyclophosphamide + Silymarin
Figure 5.5: Protective effect of aqueous extracts of *P. fraternus* and *A. marmelos* in LPO against CPA-induced hepatotoxicity. ‘a’ indicates the significant difference between the control and CPA treated groups, ‘b’ indicates the significant difference between the CPA treated and AEPF and AEAM treated groups. Each bar represents the Mean ±SE, n=6.

CPA + P : Cyclophosphamide + Aqueous extract of *P. fraternus*

CPA + A : Cyclophosphamide + Aqueous extract of *A. marmelos*

CPA + SIL: Cyclophosphamide + Silymarin
Figure 5.6: Protective effect of aqueous extracts of *P. fraternus* and *A. marmelos* in SOD and CAT against CPA-induced hepatotoxicity. ‘a’ indicates the significant difference between the control and CPA treated groups, ‘b’ indicates the significant difference between the CPA treated and AEPF and AEAM treated groups. Each bar represents the Mean ±SE, n=6.

CPA + P: Cyclophosphamide + Aqueous extract of *P. fraternus*

CPA + A: Cyclophosphamide + Aqueous extract of *A. marmelos*

CPA + SIL: Cyclophosphamide + Silymarin
Figure 5.7: Protective effect of aqueous extracts of *P. fraternus* and *A. marmelos* on haematology against CPA-induced hepatotoxicity. ‘a’ indicates the significant difference between the control and CPA treated groups, ‘b’ indicates the significant difference between the CPA treated and AEPF and AEAM treated groups. Each bar represents the Mean ±SE, n=6.

CPA + P: Cyclophosphamide + Aqueous extract of *P. fraternus*

CPA + A: Cyclophosphamide + Aqueous extract of *A. marmelos*

CPA + SIL: Cyclophosphamide + Silymarin
Figure 5.8: Representative photomicrographs of liver sections (40X, H & E). The liver section from control animals showed regular cellular architecture with distinct hepatic cells and a central vein (group I). In the AEPF, AEAM and silymarin alone treated liver showed normal hepatic cells (group II-IV). The liver sections from the toxic-induced mice (group V) showed inflammation in central vein (Star), picnotic nuclei (Arrow). The treatment of animals with CPA + AEPF (200, 300 and 400 mg/kg) and AEAM (400, 500 and 600 mg/kg) revealed a better protection of the liver architecture (group VI-XI). CPA + silymarin treated liver showed normal hepatic cells (group XII).
Table 5.1: Percentage protection of various biochemical parameters, antioxidant activities and hematological parameters after treatment with aqueous extracts of *P. fraternus* and *A. marmelos* against CPA- induced hepatotoxicity in mice.

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<th>Parameters</th>
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