Abstract

Cyclophosphamide (CPA) is a widely used antineoplastic drug, which could cause toxicity to normal cells due to its toxic metabolites. It is used to treat various types of cancer. It is a cytotoxic alkylating agent commonly used in chemotherapeutic regimens. The clinical efficacy of CPA is restricted due to its toxic effects in normal cells. Therapeutic dose of CPA causes liver disorders and nephrotoxicity which leads to gonadal toxicity, as a side effect of the drug. Therefore, it is important to prevent the oxidative stress and toxicity induced by CPA in normal cells in clinical applications.

India has a great wealth of various naturally occurring plant based drugs which have great potential pharmacological activities. Medicinal plants are sources of important therapeutic aids for alleviating human ailments. Phyllanthus fraternus is one of the medicinally important plant belongs to the family Euphorbiaceae, commonly known as “Bhuiamla”. The plant Aegle marmelos belongs to the family Rutaceae and is known as bael in Hindi. The various parts of these plants (mainly leaves and fruits) are widely used in traditional medicine for the treatment of various disorders. P. fraternus and A. marmelos have been proved for wide pharmacological potential with a great utility and usage as folklore medicines. These plants have free radical scavenging activity, therefore, may be used for the prevention and treatment of tissue damages.

In chapter I, phytoconstituents were analyzed in different plants extract by qualitative and gas chromatography- mass spectrometry (GC-MS) method. In chapter II, the study was conducted to evaluate the hepatoprotective activity of aqueous extracts of P. fraternus (AEPF) and A. marmelos (AEAM) against CPA-induced liver damage in mice. In chapter III, the nephroprotective effect of AEPF and AEAM against CPA-induced renal toxicity in mice was described. In chapter IV, the effect of AEPF and AEAM against CPA-induced changes in reproductive toxicity in male mice is explained in detail. The results of present study are summarized below:
Chapter I: Phytoconstituents in aqueous extracts of *Phyllanthus fraternus* and *Aegle marmelos*

The phytochemical screening of the extracts revealed the presence of alkaloids, saponins, tannins, flavonoids and phenols, which may have antioxidant activities. The compounds identified in GC-MS analysis of *P. fraternus* extract clearly showed the presence of pyrogallol (7.76%), chromane (8.68%), eugenol (4.72%), cerosol (6.11%), oleic acid (10.86%), carissanol (24.31%), pinane (30.62%) while in *A. marmelos*, ceneol (16.97%), limonene (19.08%), citronellol (37.85%), menthol (12.90%), piperitone (3.61%), chromane (2.20%), phthalic acid (1.83%), linolenic acid (2.32%), oleic acid (1.39%), silane (1.36%), surfynol (1.93%) was found as the major component. The presence of these constituents in the plant extracts provides the scientific evidences for the antioxidant, antimicrobial, anticancer, and anti-inflammatory properties of the plant.

Chapter II: Hepatoprotective activity of aqueous extracts of *Phyllanthus fraternus* and *Aegle marmelos* against cyclophosphamide-induced toxicity

Plant extracts and CPA (200mg/kg bw, intraperitonially) were administered to mice for 5 weeks, once a week. Silymarin (100mg/kg bw) was given as reference standard. Serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, alkaline phosphatase, acid phosphatase, bilirubin, cholesterol levels and lipid peroxidation were significantly increased (*p*<0.001), accompanied by a significant decrease (*p*<0.001) in the level of albumin in CPA-induced hepatotoxic group of mice compared to the control. However, significant amelioration in these parameters was found in AEPF and AEAM treated groups of mice. CPA treatment markedly decreased the level of superoxide dismutase and catalase in the liver as well as white blood cells and red blood cells count, which were significantly enhanced by AEPF and AEAM treatment. Histopathological examinations have also confirmed the protective efficacy of AEPF and AEAM. Results showed that extracts could protect
the liver against CPA-induced oxidative damage by possibly reducing the rate of lipid peroxidation and increasing the antioxidant defense mechanism in mice. Hence, the results of the present study revealed that AEPF and AEAM may be effective as a hepatoprotectant in CPA-induced toxicity.

**Chapter III: Nephroprotective activity of aqueous extracts of *Phyllanthus fraternus* and *Aegle marmelos* against cyclophosphamide-induced toxicity**

The kidney function markers like blood urea nitrogen and creatinine were significantly increased and a significant decrease \((p<0.001)\) was found in the kidney somatic index in CPA treated mice. However, these levels were found to be reversed in the AEPF and AEAM treated group. CPA-induced nephrotoxicity characterized by significant elevation \((p<0.001)\) of lipid peroxidation, reduced superoxide dismutase and catalase. Co-administration of AEPF or AEAM with CPA was significantly prevented the renal injury both functionally and histologically in dose dependent manner. The nephroprotective effect of AEPF and AEAM could be due to the antioxidant and free radical scavenging principles contained in the extracts. This study suggests that the extracts have nephroprotective potential, thereby justifying their ethnopharmacological uses.

**Chapter IV: Effect of aqueous extracts of *Phyllanthus fraternus* and *Aegle marmelos* against cyclophosphamide-induced reproductive toxicity**

CPA treated group showed significant decrease \((p<0.001)\) in gonadosomatic index, epididymal sperm count, sperm motility and sperm viability compared to control group, while the CPA + AEPF-treated group has significant increases \((p<0.001)\) for these variables compared to the CPA-treated group. The elevated level of lipid peroxidation by CPA was effectively reduced with AEPF. It also exhibited protective action against the CPA induced depletion of antioxidants like catalase and superoxide dismutase. Administration of AEPF along with CPA restored the histopathological architecture of testes. On the other hand AEAM treated group, the gonadosomatic
index, sperm count, motility and viability were very much reduced as well as altered the antioxidant activities, when compared to control group.

CPA used for cancer chemotherapy is known to produce toxic side-effects in multiple organs like liver, kidney and testis that were proved by biochemical and histopathological studies. The results strongly suggest that AEPF and AEAM may be considered a potentially useful candidate in combination of chemotherapy with CPA to combat oxidative stress mediated tissue injury.