Since antiquity plants have been used as a source of therapeutic agents and are playing an important role in primary health care and in the indigenous system of medicine to fight against diseases.

World Health Organization (1978) has promoted traditional medicines as safe remedies for ailments of several diseases. In past few years, a good number of studies have been carried out all over the world to prove such therapeutic efficiencies of plant extracts and phytochemicals.

Presently 25% of prescribed drugs worldwide are derived from plant sources in spite of the great progress and advancement of organic synthesis. Medicinal plants offer unlimited opportunities for the discovery of new drugs in biomedical field. Most of the natural products used in folk remedy have scientific evidences with regard to their biological activities.

Calotropis gigantea Linn. (*C.gigantea)* of family Asclepiadaceae is a perennial shrub and widely distributed in tropical and subtropical region and most abundant in Bangladesh, India, Burma and Pakistan. The root, stem, leaves, flower and latex of *C. gigantea* are reported to be utilized in traditional medicine for the treatment of toothache, ear ache, eczema, syphilis, elephantiasis, injury, pain, ulceration, epilepsy, anxiety and mental disorders. The major phytochemicals of *C. gigantea* are uscharin, calotropin, frugoside, calotroposides, amyrin, taraxasterol, β-sitosterol. Its latex rich in lupeol, calotropin, calotoxin and uscharin.

This study was carried out to collect, identify, extract, screen the group of phytochemicals and to characterize the active components present in ethanol (EECGL) and water (WECGL)

extract of *Calotropis gigantea (C. gigantea)* Linn latex extractas well as to evaluate their acute and sub-acute toxic effects inbrine shrimp , zebra fish embryo, human lymphocytes and in Swiss albino mice. The in-vitro antioxidant, anti-inflammatory, antimitotic potential of the *C. gigantea* latex extracts as well as the antineoplastic activities against Jurkat, Ehrlich Ascites Carcinoma (EAC), and Dalton Ascites lymphoma (DLA) cells were investigated.

Chemical screening analysis showed that the latex extracts of *C. gigantea* possessed flavonoids, alkaloids, triterpinoids, saponin, glycoside, cardiac glycosides. Chemical characterization studies revealed the presence of some important phytoconstituents of the plant *Calotropis gigantea.*

The LC_{50} value of EECGL and WECGL for brine shrimp (*A. salina*) was 1024 and 1280 µg/ml respectively. The two extracts at 2000 µg/ml concentration exhibited 100 % mortality in *A. salina*. Slight edema was observed on abdominal area and thoracic cavity of zebra fish embryos at 48 hpf after the treatment of EECGL and WECGL at the concentration of 2000 µg/ml. EECGL and WECGL do not show any significant change in ROS generation, DNA fragmentation in human lymphocytes. In the sub-acute repeated dose 28-day toxicity study, ethanol and methanol latex extract of *Calotropis gigantea* were administered intraperitoneally at the dose levels of 50, 100, 200, 500, 1000 and 2000 mg/kg body wt. /day. No significant ($p<0.05$) difference were observed in relative organ weights and haematological, hepatic and renal biomarkers up to the dose level of 500mg/kg body wt./day for 28 days except blood glucose and serum glutamate pyruvate transaminase (SGPT) in

comparison to the control group. No significant toxicity was seen in mice up to the dose level of 1000 mg/kg body wt. /day for 28 days in case of blood glucose and SGPT.

The *in vitro* antioxidant activity was assessed using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide, hydroxyl radical, hypochlorous acid, superoxide anion lipid peroxidation, peroxynitrite free radical scavenging method. The anti-inflammatory activity of ethanolic and water extracts was investigated by *in vitro* methods as well as in carrageenan induced mice paw edema model for acute inflammation. The findings showed that EECGL exhibited increased 2, 2-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide, hydroxyl radical, hypochlorous acid, superoxide anion lipid peroxidation, peroxynitrite free radical scavenging activities almost equal to the standard (ascorbic acid) and was far better than WECGL. EECGL showed significant HRBC membrane stabilizing activity when compared to the standard anti-inflammation drug, diclofenac sodium. EECGL and WECGL exhibited maximum 71 % and 68% inhibition in protein denaturation i.e. at 500 µg/ml. EECGL and WECGL at a dose level of 10, 20 and 50 mg/kg, prevented mice paw edema as compared to carrageenan control (at 3^{rd} and 4^{th} h) in carrageenan induced mice paw edema model while standard drug indomethacin showed prevention at the concentration of 10 mg/kg. So the results reveals that ethanolic (EECGL) and water (WECGL) extract of *Calotropis gigantea* latex possess a prominent antioxidant, anti-inflammatory activities.

EECGL and WECGL inhibited cell proliferation of Jurkat cells at the concentrations of 25 and 50µg/ml (Fig. 5.1). The IC_{50} values of EECGL and WECGL for Jurkat cells were 29.4 ±

0.667, and 43.1±0.978 µg/ml respectively producing no significant reduction in cell viability of human lymphocytes up to the dose level of 50 µg ml^{-1}. In Jurkat cell, DCF fluorescence intensity due to intracellular ROS generation was elevated by EECGL and WECGL at their respective IC_{50} doses. The extract caused pronounced chromatin condensation after PI and DAPI staining. *C.gigantea* latex extracts were found to be antimitotic in *Allium cepa* root tip cells at the concentration of 100mg/ml. EECGL treated group shows significantly less number of dividing cells compared to the control group. The mitotic index of EECGL, WECGL treated group and control group were 27%, 47% and 57% respectively. These findings also reveal that *Calotropis gigantea* latex extracts exhibit *in-vitro* cytotoxic potential against Jurkat cell and pronounced antimitotic potential in *Allium cepa* root tip cells.

The anticancer activity of ethanol and water extracts of the latex of *Calotropis gigantea* against Dalton's Ascitic Lymphoma (DLA)cells was investigated in *in vitro* and *in vivo* experimental conditions. The extracts inhibited the proliferation of DLA cells in a dose-dependent manner. Studies on cell viability, chromatin condensation using DAPI and PI staining and DNA fragmentation revealed that *Calotropis gigantea* latex extracts were capable to produce significant anticancer and apoptotic effects on DLA cells. Investigation on nitric oxide release level, reactive oxygen species formation and mitochondrial membrane potential confirmed the oxidative injury in DLA cells by *C. gigantea* latex extracts. In cell cycle analysis, DLA cell treated with the extracts of *Calotropis gigantea* induced apoptosis and there is cell cycle arrest at the G_0 /G_1 phase. To show its abilities in cancer

chemoprevention, *in vivo* studies were also done in DLA-bearing Swiss albino mice. Significant reduction in mean survival time, body weight, tumour volume and viable tumour cell count were observed. Surprisingly EECGL and WECGL had no toxic effects on normal lymphocytes at doses up to 50 μg ml^{-1}. The results of this investigation clearly demonstrate that the extracts of *Calotropis gigantea* inhibit the cell proliferation and survival of DLA cells via oxidative injury and induction of apoptosis in a dose-dependent manner.

Cancer is the one of the deadly diseases and it is the third leading cause of death worldwide. The roots and leaves of *Calotropis gigantea* are used traditionally for the treatment of abdominal tumours. The present study designed to evaluate the efficacy of *Calotropis gigantea* latex extracts (EECGL and WECGL) from as anticancer agents against Ehrlich Ascites Carcinoma in comparison to EECGL. Study of cytotoxicity, cell viability, cell morphology, chromatin condensation, DNA fragmentation, nitric oxide (NO) generation and release level revealed that EECGL and WECGL possess antiproliferative and apoptotic potential on EAC cell line at IC_{50} of 25 and 40.98 μg mL^{-1}. EECGL and WECGL were administered intraperitoneally at the dose level of 100- and 200 mg/kg body weight/day respectively for 14 consecutive days after 24 hour of EAC cell inoculation (1×10^6 cell) to mice using 5-fluorouracil as standard drug. Mean survival time, tumor volume, haematological and oxidative stress related parameters were studied. Decrease in tumor volume, and body weight of the EAC-bearing mice were observed in EECGL and WECGL-treated mice group compared to EAC-control mice. Treatments with EECGL and WECGL

were associated to decrease in the levels of lipid peroxidation (MDA), and increase in the levels of reduced glutathione (GSH), and antioxidant enzymes activities. Upregulation of proapoptotic proteins as well as downregulation of antiapoptotic proteins involving intrinsic apoptotic pathway were detected. Immunohistochemical observations from treated solid tumors also established the antineoplastic and antiangiogenic potentiality of*Calotropis gigantea* latex. From the present investigation, it is established that both of EECGL and WECGL of *Calotropis gigantea* latex possess *in vitro*cytotoxic, apoptotic, *in vivo* antineoplastic, antioxidant activities against EAC cells but the efficacy of EECGL is more than WECGL. The findings from in vitro and in vivo studies have led the idea that EECGL and WECGL could be potent antineoplastic drugs in future.