SUMMARY AND CONCLUSION

Cancers are a group of diseases, characterized by uncontrolled proliferation of cells. World Health Organization stated that cancer occupies the second position in the list of killer diseases in the industrially advanced countries while in the developing countries; it ranked as the fifth (WHO, 1990). This disease is multifactorial in origin and may develop due to radiation exposure, exposure to chemicals, viral infections and due to genetic alterations. The disease has a treatment regime consisting of radiation, chemotherapy, surgery, gene therapy etc. Although curative treatments are available for some cancers including childhood leukemia and testicular cancer, many metastatic solid tumours are not sensitive to current therapies. An intensive search is being made to develop a new drug with minimum side effects to counteract this disease.

The vast biodiversity of nature might provide bioactive compounds that may be useful in the fight against cancer. Many of the important and effective drugs used to treat cancer are derived from higher plants. Hence the present study was conducted to isolate tumour reducing components from a selected medicinal plants i.e. Cassia fistula L.

The study comprised of in vitro as well as in vivo experiments. In the first part of the study in vitro screening of 10 medicinal plants were carried out to find out the cytotoxicity of their aqueous extract. Out of these 10 medicinal plants the aqueous extract of dried powdered bark of C fistula L showed maximum cytotoxicity against murine cell lines namely DLA and EAC. So it was selected for further studies. Different organic extracts of the said material was prepared by differentially extracting it with organic solvents of different polarity and cytotoxicity of each one of them was assayed against DLA and EAC cell lines by a short-term cytotoxicity assay. In this assay methanolic
extract of *C. fistula* or MEC showed maximum cytotoxicity. Therefore it was selected for further studies. The long term cytotoxicity and antiproliferative activity of the MEC was then assayed by a tetrazolium assay namely MTT, using a panel of 5 human cancer cell lines namely MCF7, SW 480, HCT 116, HeLa and IMR32. This was done in a dose and time dependent manner. The MEC showed maximum activity towards MCF7, HCT116 and IMR 32.

In the next part of the experiment Ascites and solid tumours were induced in Balb/c strain of mice by transplanting DLA and EAC cell lines. The Ascites tumour models, which are also termed as ‘test tube in a mouse’, provided important information about the tumour reducing properties of MEC. MEC prolonged the life span of tumour bearing animals, reduced the viable tumour cells in the peritoneal fluid and reduced the increase in body weight due to tumour burden in all tested concentrations. The next part of the *in vivo* studies was consisted of experiments to analyze the solid tumour reducing properties of MEC. The MEC could reduce the tumour volume of transplanted EAC and DLA solid tumours. MEC also reduces the spleenic swelling due to tumour. It was also observed that haematological parameters namely Hb, RBC, WBC and DC, which were changed significantly in untreated control mice, returned to normal range in most of the drug treated groups. Similarly it was also found that the extract could reduce the hepatotoxicity and nephrotoxicity caused by tumour burden. This was evident from the fact that the extract normalized SAKP, SGPT and SGOT in *i.l* treated group. This study also revealed that *Cassia fistula* L. possessed potent antioxidant properties. In cancerous condition, the activity of SOD, GR, and GSH were decreased. The activity of three enzymes was effectively elevated by the application of drug when compared to the tumour control mice. Pathological conditions have direct correlation with increased lipid peroxidation. The level of TBARS increased in tumour cell line bearing mice, which on administration of the drug decreased considerably.
Out of the two \textit{in vivo} models tested, the lymphoma model (DLA) was more sensitive to MEC than the carcinoma model (EAC). The mode of the administration of the MEC was also important. The intraperitoneal mode of administration was found to be less effective as compared to intralesional mode of administration where the MEC was directly injected to the tumour. As the final stage of the \textit{in vivo} studies the chemopreventive potential of MEC was studied on murine two-stage skin chemical carcinogenesis model where mouse skin papilloma were induced by DMBA and promoted by croton oil. The most effective dose of the MEC in other experiments namely 100mg/kg body weight was selected for this experiment. MEC was applied in preinitiation stage, post initiation stage and promotional stage. MEC was able to reduce papilloma formation in all the treated groups. But maximum effect was shown in reducing papilloma formation in promotional stage. It also reduces the latency period and no. of animals developing papilloma compared to control. Further MEC applications fail to induce any change in body weight in the treated animals.

In the final part of the study, the apoptogenic effect of MEC was estimated in cultured human cancer cell lines, by DAPI assay, Mitosensor assay and caspase assay. DAPI assay was performed to visualize the morphological changes in cells treated with MEC. The MEC treated cells showed nuclear condensation or pyknosis indicating apoptosis. Similarly mitosensor assay proved MMP and Cytochrome C release in MEC treated cell lines, which is an indication of apoptosis. The MEC did increase the total cellular caspase activity in treated cell lines, which also showed that MEC could induce apoptosis. Hence the mechanism by which MEC impart its antitumour activity might be by the induction of apoptosis in cancer cells.