6 SUMMARY AND CONCLUSIONS

6.1 Summary

Genotoxic agents are substances capable of causing direct damage to genetic material, leading to mutation and clastogenic disturbances, which further can lead to occurrence of degenerative diseases and cancer. Reduction in the incidence of such damage can lead to reduction in the occurrence of mutation based diseases. Exposure to environmental pollutants, industrial chemicals and effluents, heavy metals, cytotoxic drugs and other genotoxicants might result in the development of different kinds of cancer. Toxicity of substances is manifested in diverse forms like DNA damage, formation of micronuclei, Chromosomal aberrations, cell death or abnormal cell growth leading to tumour formation. Contact with these agents is bound to happen time and again and creates a threat to human health. Cancer chemotherapy is one of such situations where toxicity to non cancerous tissues is a major problematic issue. The need to overcome the genotoxic side effects of chemotherapeutic as well as other drugs is impending. Use of natural compounds with antioxidative, antigenotoxic, antimutagenic and cytoprotective abilities has gained importance globally. Understanding the inherent genotoxic/antigenotoxic as well as cytotoxic/cytoprotective of these natural substances is of utmost importance, to employ these substances for their chemotherapeutic and chemopreventive applications. With this background, we attempted to evaluate the genotoxic and antigenotoxic properties and also the cytotoxic/cytoprotective abilities of two plant active constituents namely apocynin and diosgenin, in this thesis. Apocynin is a strong inhibitor of the enzyme NADPH oxidase and is bestowed with several pharmacological applications. Diosgenin also, has well known therapeutic applications. Reports on the antigenotoxic properties and cytoprotective abilities of these two compounds are limited. Hence, we studied these two compounds.

The incidence of micronuclei in the polychromatic erythrocytes of mice bone marrow was studied, as an indicator of genotoxic damage inflicted on the DNA by external agents. The chemotherapy agent cyclophosphamide was employed to induce genotoxic conditions in vivo. Two biomarkers of oxidative stress, namely cellular status of lipid peroxidation and reduced glutathione in the hepatic tissues were analysed since
oxidative stress is a major mechanism of genotoxicity. Higher levels of oxidative stress are conducive for genotoxic damage. The influence of the test compounds on the total count of WBC, RBC and haemoglobin content was also studied. The efficiency of the test compounds in terms of cytotoxicity and cytoprotective/anticytotoxic nature was established by performing MTT assay with two different types of cell lines, CHO-K1 and HepG2, to ascertain their safety, against the cytotoxicity induced by chemotherapy drug cisplatin. The protective abilities of the plant compound apocynin against oxidative DNA damage was also studied by agarose gel electrophoresis. Apocynin was also tested for any inherent haemolytic properties. The effect of the selected plant compound apocynin on occurrence of chromosomal aberrations in the root tip meristems of *Allium cepa*, as an indicator of genotoxic and antigenotoxic properties, in the presence of suitable negative and positive controls, was also studied.

**In the various experimental studies we carried out, Apocynin showed –**

- Significant antigenotoxic activity in the mice bone marrow micronucleus assay by reducing Mn frequency induced by cyclophosphamide.
- Efficient restoration of total WBC count against CP induced luecopenic conditions in mice.
- Protection against lipid peroxidative damage and increase in cellular antioxidant GSH in the liver homogenates.
- Significant reduction in the chromosomal aberrations induced by CP in *Allium* root meristems.
- Exhibited no inherent cytotoxic activity towards both HepG2 and CHO-K1.
- Countered the cytotoxicity of cisplatin in cell lines HepG2 and CHO-K1. It did not show any selectivity towards the normal cells in its cytoprotective action.
- Protection against oxidative DNA damage caused by cisplatin *in vitro*.
- Showed no haemolytic activity, indicating it to be safe on the blood system.

**Our study also revealed that diosgenin shows –**
- Significant antigenotoxic activity in the mice bone marrow micronucleus assay by reducing Mn frequency induced by cyclophosphamide.
- Efficient restoration of total WBC count against CP induced luecopenic conditions in mice.
- Protection against lipid peroxidative damage and increase in GSH in the liver homogenates.
- Significant cytotoxicity towards HepG2 cells in culture but no cytotoxicity towards CHO-K1 cells.
- Significant enhancement of cytotoxic action of cisplatin on cancer cell line HepG2 but does not affect the cytotoxicity of cisplatin on CHO-K1. It does not exert any cytoprotective effect towards both the cell lines.

6.2 Conclusions

- The results of this study indicate the potential antigenotoxic and anticytotoxic efficiency of apocynin under the studied experimental conditions.
- With careful further studies, apocynin has the potential to be employed as antigenotoxic/chemopreventive agent in chemotherapy strategies, to prevent clastogenic damages induced by chemotherapeutic drugs. It also does not pose any cytotoxic or genotoxic risk.
- Our findings point that diosgenin is bestowed with promising qualities as an antigenotoxic agent that need further exploration and make it a potential candidate for application as an adjuvant in the chemotherapy strategies to manage the clastogenicity of drugs.
- Our observation that diosgenin has cytotoxicity only towards cancer cells and not towards normal cells makes it a potent candidate for development into a therapeutic agent.
6.3 Future perspectives

- The exact mechanism of action of apocynin and diosgenin in the nulling the cyclophosphamide induced genotoxic effects be interplay of molecular mechanisms which need to be elucidated further.

- Apocynin did not show any selectivity between the two studied cell lines. It showed similar protective effects towards both the cancer and normal cell lines. It is necessary to further elucidate this aspect by further studies, employing different cell lines and experimental conditions.

- The selective cytotoxicity of diosgenin need to be confirmed with multiple cell lines and experimental conditions.