CHAPTER - 6

SUMMARY AND CONCLUSION
Cancer Chemoprevention is an important practical strategy for management of cancer and may be defined as the administration of synthetic or natural agents to prevent the initiational and promotional events that occur during the process of neoplastic development (Boone et al., 1990). Chemoprevention research evaluates ways and means to reduce the risk and incidence of cancer (Kelloff et al., 1990). Cancer prevention involves the intervention with dietary constituents and plant derived products such as vitamins, minerals and other micronutrients to reverse, suppress or prevent the process of carcinogenesis (Hakama, 1998). Epidemiological studies suggest that diet, nutrition and lifestyle play a significant role in the pathogenesis of cancer (Notani, 2001). The Chemoprevention branch of National Cancer Institute has developed strategies to identify and characterize naturally occurring agents possessing anti-carcinogenic activity (Boone et al., 1990).

Central to defensive mechanism against toxic chemicals is xenobiotic metabolism i.e. activation and detoxification. This involves Phase I and Phase II enzymes and the
anti-oxidant enzymes. The International Union against cancer and several other organizations have stressed on the fact that modulation in the activities of expression of these enzymes by chemopreventive agents or phytochemicals is an important step in the mechanism of chemoprevention (Hakama, 1998). The anti-oxidant responsiveness of phytochemicals has been well established by several studies on animal models (Singh et al., 2000; Rekha et al., 2001; Singh et al., 2001).

Phase I enzymes i.e., Cyt b₅ and Cyt P₄₅₀s, activate xenobiotic compounds to their reactive forms, either by oxidation or reduction reactions, thus preparing it for Phase II metabolism. Most important Phase II enzymes participating in the detoxification process are GST isozymes. It acts by facilitating the conjugation of reactive metabolites to cellular biomolecules such as GSH, thereby converting them to more polar products that assist in their excretion from the body (Williams, 1959).

Anti-oxidant enzymes mitigate reactive oxygen species from the body formed due to oxidative stress. GSH prevents oxidation of cellular protein and also directly detoxifies
reactive oxygen species (Ketterer, 1998). GR helps in maintaining the basal level of GSH (Lopez-Barea et al., 1990). SOD scavenges superoxide anion radicals (Geetami et al., 1998), the only enzyme known to use free radicals as a substrate. However, the free radical scavenging activity of SOD is effective only when it is followed by increase in the activity of CAT and/or GPX since SOD generates H$_2$O$_2$ as a metabolite, which is toxic and has to be scavenged by CAT or GPX (Sancheti et al., 2005).

The Chemopreventive property of the hydro-alcoholic extract of a fruit, *S. cumumi*, Skeels (Fam: Myrtaceae), a vegetable, *M. oleifera*, Lam (Fam: Moringaceae) and a medicinal plant, *P. urinaria*, Linn (Fam: Euphorbiaceae) on drug metabolizing enzymes have been evaluated in the liver of Swiss albino mice (6-8 weeks old).

The effect of two doses (50μl and 100μl/mouse/day of the fruit extract of *S. cumuni* and 125 mg and 250 mg/ kg body weight/day of *M. oliefera* and *P. urinaria*) were investigated on drug metabolising Phase I (Cyt b$_5$ and Cyt P$_{450}$) and Phase II (GST) system enzymes, anti-oxidant enzymes, glutathione content and
lipid peroxidation in the liver of female Swiss albino mice for 7 and 14 days respectively. Butylated hydroxyanisol (BHA) fed at a dose of 0.75% in diet for 7 and 14 days was used as a positive control to validate the assay protocols. Acid microsomal fractions were prepared by the method of Fry and Bridges (1975). The activity of Cyt P450 and Cyt b5 were assayed in microsomal suspension by the method of Omura and Sato (1964). The cytosolic GST activity was determined by the method of Habig et al. (1974). The GSH content was estimated by the method of Moron et al. (1979). The activity of GPx, GR, SOD, CAT and LPO levels were measured by the method of Paglia and Valentine (1967), Carlberg and Mannervik (1985), Marklund and Marklund (1974), Aebi (1984) and Varshney and Kale (1990) respectively. The microsomal and cytosolic protein contents were estimated by the method of Lowry et al. (1951). All results are expressed as mean ± SD of 8-10 animals. Statistical significance of differences between the control and the treated groups were determined by ANOVA (Analysis of variance) test.
In the *S. cumuni* treated groups, significant dose dependent increase ($P< 0.01$) in the activities of Cyt P450, Cyt b$_5$, GP$_X$, SOD and CAT and dose and time dependent increase of GST, GSH and GR levels were observed. The LPO levels showed significant dose dependent decrease ($P< 0.01$) at both the dose levels of treatment when compared with the control values.

The *M. oleifera* treated groups exhibited a significant dose and time dependant increase ($p<0.05$ to $p<0.01$) in the activities of hepatic Cyt b$_5$, GSH, GPx, SOD and a dose dependent increase in Cyt P450, GST, GR and CAT activity. A significant dose dependent decrease ($P<0.01$) in the hepatic LPO levels were observed at both the dose levels of treatment when compared with the control values.

A dose and time dependant increase ($P< 0.05$ to 0.01) in the activities of Cyt b$_5$, GST, GSH, SOD and a dose dependent elevation in the Cyt P450, GR, GPx and CAT activities were observed in the *P. urinaria* treated mice with both the doses of the extract. The LPO levels were found to be decreased significantly.
(P< 0.01) in all the treated groups in comparison to the control value.

BHA treated groups (positive control) exhibited significant increase (P< 0.01) in the activities of hepatic Phase II detoxification system enzymes, anti-oxidant enzymes and GSH and decrease in LPO level.

Further, the chemopreventive efficacy of the modulators have been evaluated on two-stage process of skin carcinogenesis in 6-7 weeks old female albino mice induced by a single application of 7,12-dimethylbenz (a) anthracene (50µg/50µl of acetone), and two weeks later, promoted by repeated application of Croton oil (1% in acetone/ three times a week) till the end of the experiment (15 weeks) to quantitate the chemopreventive response. The extracts were topically applied on the shaven backs of the mice at a dose of 5mg/kg body weight/day for 15 weeks at the peri-initiaational stage (i.e., 7 days before and 7 days after DMBA application), promotional stage (i.e., from the time of Croton oil application) and both peri- and post-initiaational stages (i.e., 7 days prior to DMBA application and continued till the end of the
experiment). The significant level of difference between control and experimental values were statistically analyzed using chi-square test at 5% probability level.

In the DMBA-induced skin papillomagenesis study the inhibition of tumor incidence recorded a significant reduction in all the modulator treated groups in comparison to the control (i.e., the mice treated with DMBA and Croton oil only). The study has revealed a significant decrease (P<0.05) in the cumulative number of papillomas, average number of papillomas per mouse, percentage of mice with papillomas and papilloma per papilloma bearing mouse when the animals received a topical application of the extract as compared to control group. The percentage inhibition of tumor multiplicity is found to be increased significantly (P<0.05) in all the treated groups in comparison to the control groups. The latency period was also found to be extended notably during the experiment that might be permissible for the protective activity of the extract during the progression and/or promotion stages of tumorigenesis.
Thus the findings are suggestive of a possible chemopreventive activity of *S. cumuni*, *M. oliefera* and *P. urinaria*. However, the mechanisms underlying the action of the modulators for their chemopreventive efficacy or the role of any constituent(s) of the extracts in mediating the chemopreventive response needs further investigation. Future research in the area of chemoprevention by plants/plant products offer great hope for making significant progress for cancer control and holds promise for huge public health benefits which is a challenge to the scientific community.