CHAPTER 6

SUMMARY

AND

CONCLUSION
Cancer continues to represent the largest cause of mortality in the world and claims over 6 million lives every year (Abdullev et al., 2000). An extremely promising strategy for cancer prevention today is chemoprevention, which involves the intervention with dietary constituents, plant derived products and other micro nutrients to reverse, suppress or prevent the process of carcinogenesis (Hakama, 1998). Keeping this in view, recently medicinal plants and their active principles have received growing attention as potential chemopreventive agents as depicted by various studies (Lee and Park, 2003; Sancheti and Goyal, 2006; Meena et al., 2006).

In the present study, the chemopreventive efficacy of the Eclipta alba extract have been evaluated on two stage process of skin carcinogenesis in 6-8 weeks old female albino mice induced by a single application of 7, 12-dimethylbenz (a) anthracene 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 extract was topically applied on the shaven backs of the mice at a dose of 5 mg/ kg body weight /day for 15 weeks at the peri-initiational 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 initiational stages (i.e., 7 days prior to DMBA application and continued till the end of
the experiment). The significant level of difference between the control and experimental values were statistically analyzed using student's t test at 5 % probability level.

In addition, the histological change in the tissues of the affected region of the skin was also examined by preparing permanent slides by the method of Kehar and Wahi (1967).

In the DMBA induced skin papillomagenesis study the inhibition of tumor incidence recorded a significant reduction in all the modulator treated groups (Groups II, III and IV) in comparison to the control (i.e., the mice treated with DMBA and croton oil). 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 papillomas per papilloma bearing mouse when the animals received a topical application of the extract as compared to the 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 histopathology of the skin showed visible reduction in the size of the tumors in comparison to the control. The modulator treated groups (Group II, III and IV) also have not exhibited any distinct core tissues.
Further the chemopreventive efficacy of the *Eclipta alba* extract in the activities of hepatic biotransformation enzymes i.e., Phase I (Cytochrome P<sub>450</sub> and Cytochrome b<sub>5</sub>), Phase II enzyme (Glutathione S-transferase), the antioxidant enzymes (i.e., SOD and CAT) and lipid peroxidation was evaluated at a dose level of 125 mg / kg body weight and 250 mg / kg body weight for 14 days respectively. This is because the International Union against cancer and several other organizations have stressed on the fact that a modulation in the activities of the expression of these enzymes by chemopreventive agents or phytochemicals is an important step in the mechanism of chemoprevention (Hakama, 1998).

Two classes of enzymes have been identified for the metabolism of xenobiotic compounds to excretory metabolites known as Phase I and Phase II enzymes (Williams, 1971). The Phase I (Cyt P<sub>450</sub> and Cyt b<sub>5</sub>) enzymes begin the process of biotransformation by oxidizing, reducing or hydrolyzing toxins, creating biotransformed intermediates and the phase II (GST) enzymes perform conjugation reactions which help to convert the biotransformed intermediates from phase I to less toxic water soluble substances that are easily excreted or eliminated from the body (Percival, 1997).

The antioxidant enzymes mitigate reactive oxygen species and protect the body from the deleterious effects of oxidative stress (Pugliese, 1998).
GSH directly quenches reactive hydroxyl free radicals, other oxygen-centered free radicals, and radical centers on DNA and other biomolecules and it is also the biochemical foundation of P_{450} detoxification in the liver, kidneys, lungs, intestinal epithelia, and other organs (Kidd, 1997). SOD protects the cell from the injurious effects of superoxides (Bandyapadhyay et al., 1999). Catalase protects the cells from the toxic effects of hydrogen peroxide by catalysing the conversion of hydrogen peroxide (H_{2}O_{2}) to water and molecular oxygen (Gaetani et al., 1996).

In the present study the effect of two doses (125 mg/kg body weight/day and 250 mg/kg body weight/day of Eclipta alba) was investigated on phase I enzymes, phase II enzyme, antioxidant enzymes, glutathione content and lipid peroxidation in the liver of female Swiss albino mice for a period of 14 days. Butylated hydroxyanisol (BHA) fed at a dose of 0.75% in diet for 14 days was used as a positive control to validate the assay protocol. Acid microsomal fractions were prepared by the method of Fry and Bridges (1975). The activity of Cyt P_{450} and Cyt b_{5} were assayed in microsomal suspension by the method of Omura and Sato (1964). The cytosolic GST activity content was estimated by the method of Habig et al. (1974). The GSH content was estimated by the method of Moron et al. (1979). The activity of SOD was
measured by the method of Marklund and Marklund (1974) and that of catalase was measured by the method of Aeibi (1984). The activity of lipid peroxidation (LPO) was measured by the method of Varshney and Kale (1990). The microsomal and cytosolic protein contents were estimated by the method of Lowry et al. (1951). All the results are expressed as mean ± SD of 10 animals. Statistical significance of differences between the control and treated groups were determined by the Student’s t test.

In the modulator treated groups (Group II, III and IV), there was a significant dose dependent increase (P<0.01) in the activities of Cytochrome P450 and Cytochrome b5 in contrast to the control (Group I). The glutathione S transferase, reduced glutathione, superoxide dismutase and catalase activity also exhibited a significant increase (P<0.01) for both the low and high doses when treated for a period of 14 days. The lipid peroxidation levels showed a significant dose dependent decrease (P<0.01) at both the dose levels when compared 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 (GST), antioxidant enzymes (SOD and CAT), GSH and decrease in LPO level.
Thus the findings in the present study are suggestive of a probable chemopreventive activity of *Eclipta alba*. However, the mechanisms underlying the action of the modulator for its chemopreventive efficiency or role of any of the constituents of the extract in mediating the chemopreventive response needs auxiliary investigation. Further delve into the area of chemoprevention by plant or plant derived products offer great hope for making noteworthy progress for cancer control and holds promise for huge public health benefits which is a challenge to the scientific community.