CHAPTER 7

SUMMARY AND CONCLUSION
Oxygen free radicals play an important role in the etiology of various diseases, especially cancer, inflammation, aging, vascular disorders etc. (Cerutti, 1985; Kensler and Taffe, 1986; Halliwell and Gutteridge, 1985). Antioxidants act as a major defence against free radical mediated toxicity by protecting membrane and cytosolic components against damage caused by these highly reactive species. Curcumin, the active ingredient of turmeric is a well known antioxidant (Elizabeth and Rao, 1990; Sharma, 1976) and has been reported to possess antitumour (Soudamini and Kuttan, 1988), anticarcinogenic, (Huang et al, 1988), antimitagenic (Nagabhushan et al, 1987), antiinflammatory (Srimal and Dhawan, 1973) and antibacterial (Ramaprasad and Sirsi, 1956) activities.

Phenolic structure of curcumin together with the $\beta$-diketone structure is supposed to be responsible for its high biological activity (Huang et al, 1991). It would be of interest to find out the biological activity of compounds similar to curcumin having different ring substitutents. With this rationale a number of curcumin like compounds were isolated and synthesized and their biological activity determined. These included 3 natural curcuminoids, 8 synthetic curcuminoids, 19 chalcones, 12 sydnone substituted chalcones and 10 structurally related compounds.
All the natural curcuminoids were found to be cytotoxic in the short term in vitro assay and in tissue culture. Though curcumin III showed maximum activity in the short term assay, all the three curcuminoids showed similar activity in tissue culture. Except salicyl curcuminoid synthetic curcuminoids did not have a significant activity in the short term assay but were found to be very active in tissue culture.

All the three natural curcuminoids produced an increase in life span of ascites tumour bearing mice. Curcumin III was found to be the most active among the three. Most of synthetic curcuminoids were also found to increase the life span of ascites tumour bearing mice and veratryl curcuminoid showed maximum activity. Natural curcuminoids were also studied for solid tumour reduction and all the three showed a significant reduction of solid tumour growth. However administration of curcuminoids did not prevent the tumour associated death of the animals.

All the three natural curcuminoids were found to inhibit lipid peroxidation and curcumin III was found to be the most effective. Among synthetic curcuminoids studied for lipid peroxidation inhibiting activity, salicyl and piperonal curcuminoids showed maximum activity.

Among natural curcuminoids, curcumin III was found to be a better scavenger of superoxide radicals. When curcumin III showed a 50% inhibition of superoxide production at a
concentration of 1.9 ug/ml, 4.25 ug/ml curcumin II and 6.25 ug/ml curcumin I was required for obtaining the same result. Among synthetic curcuminoids, salicyl, p-anisyl and piperonal curcuminoids were found to be potent inhibitors of superoxide production.

All the three natural curcuminoids were found to be potent scavengers of hydroxyl radicals. Among synthetic curcuminoids salicyl, piperonal, and p-anisyl curcuminoids were found to be effective scavengers of hydroxyl radicals.

The curcuminoids were very effective in inhibiting the superoxide production by activated macrophages. Curcumin I, curcumin II and curcumin III produced an inhibition of 56.5%, 51.1% and 66.1% respectively to the in vivo superoxide production, at a dose of 50mg/Kg body weight. Synthetic curcuminoids also inhibited superoxide production in vivo, among which salicyl and veratryl curcuminoids showed maximum activity.

The curcuuminoids were also studied for antimutagenic and anticarcinogenic activity. Among natural curcuminoids, curcumin III was found to be the most effective antimutagen. Curcumin I and curcumin II also showed antimutagenic activity. Among synthetic curcuminoids, salicyl curcuminoid was found to be the most active antimutagen followed by piperonal, p-anisyl, furfural and veratryl curcuminoids.
All the natural curcuminoids, especially, curcumin II and curcumin III were found to be potent inhibitors of carcinogenesis induced by DMBA and croton oil. Among synthetic curcuminoids salicyl curcuminoid showed significant anticarcinogenic activity.

Antiinflammatory and antibacterial studies were also conducted using the curcuminoids. All the natural curcuminoids and most of the synthetic curcuminoids showed significant antiinflammatory activity as measured by the casein induced mice paw oedema method. Curcumin III and Salicyl curcuminoid were found to be the most potent antiinflammatory agents.

The antibacterial activity of the curcuminoids were studied using gram positive and gram negative bacteria and curcumin III and salicyl curcuminoids were found to have significant antibacterial activity which was found to be increased in the presence of light.

A number of chalcones, sydnone substituted chalcones and structurally related compounds which have a close structural similarity to curcumin were also screened for their cytotoxic, tumour reducing and antioxidant activities. All the chalcones tested were found to be cytotoxic in the short term assay and in tissue culture. Sydnone substitution was found to increase the biological activities of the chalcones.
Methyl derivative of sydnone substituted chalcone produced maximum tumour reducing activity. Though most of the chalcones were found to be good antioxidants, their tumour reducing activity was rather low.

These observations suggest that the activity of these curcuminoids is essentially due to the phenolic group which can react with a free radical to form the phenoxy radical. The high activity of these compounds may also be due to the presence of a double bond in conjugation with the phenyl ring through which the stability of the phenoxy radical is increased by electron delocalization.

All the natural curcuminoids and the synthetic curcuminoids such as salicyl, piperonal and p-anisyl curcuminoids were found to be potent scavengers of oxygen free radicals. The antioxidant activity of these compounds may be the main contributing factor to their antitumour, antimutagenic and anticarcinogenic activity. Several procarcinogens are activated to ultimate carcinogens by the reactive oxygen species (Dix and Marnett, 1983; Marnett, 1987). Curcuminoids being potent antioxidants may be scavenging these free radicals there by inhibiting the activation of carcinogens.

Piperonal, veratryl and p-anisyl curcuminoids showed good activity in the in vivo experiments, even though their activity was low in some of the in vitro experiments. This
may be due to the metabolic conversion of the curcuminoids to the phenolic form, taking place \textit{in vivo}. Similarly these curcuminoids which showed excellent antimutagenic activity did not show significant anticarcinogenic activity again confirming the fact that conversion to hydroxyl derivative is a prerequisite for the anticarcinogenic activity as the curcuminoids are applied topically in the latter case. Antiinflammatory and antibacterial studies also support this hypothesis.

In conclusion, most of the curcuminoids and chalcones used in the present study are good antioxidants and are capable of protecting against the toxicities induced by reactive oxygen species and by certain xenobiotic. Curcumin III and its ortho isomer, salicyl curcuminoid were found to be the most active compounds studied in the present work. As turmeric which is the natural source of curcuminoids has been found to be non-toxic, non-teratogenic and non-carcinogenic (Bille et al, 1985; Wahlstorm and Blennow, 1978), its role in chemoprevention is again confirmed. However curcumin has been accepted by NCI as a chemopreventive agent (Kelloff et al, 1994).