CHAPTER : VIII

GENERAL DISCUSSION
Curcumin a major yellow pigment in turmeric is widely used as a spice and colouring agent in several foods as well as cosmetics and drugs. A wide range of biological and pharmacological activities of curcumin has been investigated (Govindarajan, 1980; Huang et al., 1992). Curcumin is a potent inhibitor of mutagenesis and chemically induced carcinogenesis (Azuine and Bhide, 1992; Rao et al., 1995; Nakamura et al., 1998).

Its antioxidant potential as well as its relatively low toxicity to rodents is currently attracting strong attention. Curcuminoids exhibited antioxidant activities in some in vitro lipid peroxidation system and suppressed induction of hydrogen peroxide production and oxidized DNA formation in mouse epidermis (Huang et al., 1997). Curcumin inhibits neutrophil responses and superoxide generation in macrophages (Joe and Lokesh, 1994). Another major colourless metabolite of curcumin in the form of glucuronide conjugate had stronger antioxidant activity.

There has been an increasing interest in the protective function of dietary antioxidants, which are also candidates for cancer chemo prevention. However, it has been pointed out that one component in these antioxidants is not enough to prevent carcinogenesis. There are few data concerning the role of curcumin on the status of mixed function oxygenase enzymes and antioxidant function during carcinogenesis in vivo. Furthermore, there is a controversy as to the effectiveness of these molecules.

The antioxidant profile probed in the present study for curcumin interaction in the metabolism during cholanthrene induced carcinogenesis is represented by the enzyme triad superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT).
The general character of trend in changing activity of the individual enzyme during the experimental period under conditions of 3-methylcholanthrene (3MC) exposure, curcumin exposure and combination of 3-methylcholanthrene and curcumin exposure is projected with highly significant (p<0.001) depression of antioxidant activity by 3MC and significant elevation by curcumin which is modulated on either side by presence of curcumin with 3MC, maintaining an intermediate position between the two extremes (Fig.IV.2, 4 and 6).

The observed general trend as discussed in detail in the chapter IV is authenticated by the trend analysis presented in (Fig.VIII. 1). It is clearly evident that the antioxidant enzyme activity in the overall metabolism in toto is most elevated with curcumin administration with uniform gradient upto 45th days of daily administration which finally acquires as almost steady state after 60th days and is maintained with reasonable uniformity upto the end of experiment on 120th day as indicated by a 'R' value of 0.9891 obtained through the second order polynomial regression. In the same set of analysis it is observed that the antioxidant status is depressed by about 20 percent from the control situation by 3MC and the effect is maintained upto termination of experiment on 120th day with a 'R' value of 0.6251. On combined exposure to 3MC and curcumin the antioxidant status is observed to fluctuate minimally around the control base line throughout the entire experimental period indicating certain amount of buffering activity of curcumin on 3MC depleted antioxidant status or inhibitory effect of 3MC induced carcinogenic metabolic trend on curcumin inducible augmentation of antioxidant enzyme system.
Fig. VIII.1: Trend of antioxidant enzymes during experimental period with curcumin, 3-methylcholanthrene and combination of 3-methylcholanthrene and curcumin.
The mixed function oxygenase (MFO) enzyme system in the present study is represented by the triad of aryl hydrocarbon hydroxylase (AHH), cytochrome P450 (CYP) and xanthine oxidase (XOD). The effect of experimental condition on the resultant functional trend of MFO activity is represented graphically in the (Fig.VIII.2). The overall status of MFO activity in different phases of the experiment as deduced from the different tissue samples summarily represent that the MFO function is most elevated with an increase of above 250 percent of base line control by 3MC which is more pronounced with increase in duration. In contrast to the magnitude of 3MC induced elevation of MFO activity, curcumin induces the enzyme activity only about 25 percent from the base line which may suggest that 3MC is 10 times more potent that curcumin in enhancing the MFO system. It is interesting to observe that the character of curcumin induced enhancement of MFO function and 3MC induced enhancement have noticeable differences with relation to duration of exposure. Although 3MC is administered as a single dose the initial enhancement is followed by an augmenting trend, which further increases upto the terminal phase of experiment on 120th day. But the curcumin induced elevation is uniform throughout the entire period which runs parallel to the base line (Fig.VIII. 2) and is maintained by daily curcumin administration in contrast to an initial single dose of 3MC. The readjustment of MFO function to meet the metabolic need under 3MC triggered alteration superimposed by daily curcumin exposure presented by the character of the MFO function trend line under the experimental condition suggest that curcumin plays some form of definite role in back regulation of 3MC induced MFO enhancement towards normalization. The observed role of curcumin over a 3MC induced metabolic change may explain a fraction of the probable mechanism through which curcumin exerts its chemopreventive function as an anticarcinogen. From the comparative
Fig. VIII. 2: Trend of mixed function oxygenase enzymes during experimental period with curcumin, 3-methylcholanthrene and combination of 3-methylcholanthrene and curcumin.
interpretation of the regression trends between MFO functions under 3MC and 3MC and curcumin combination exposure, it may be suggested that presence of a sustained level of curcumin maintained through daily administration under the present experimental set up enhances oxidative degradation of the initial cholanthrene dose as a factor of curcumin induced enhancement of MFO function and thereby prevents the increase in 3MC induced MFO function reducing the effective half life of 3MC in an exponential rate. This suggestion may be supported by the almost identical ‘R’ values of the trend line with a reversal in direction giving a mirror image of each other. These observations totally tally with the reports and experimental findings that the time lag between exposure to 3MC and detection of carcinoma is significantly reduced with higher initial doses (Baruah, 2000).

On a concerted observation over the trends of metabolic adjustment with changing antioxidant enzyme and MFO enzyme profile under the present experimental setup with 3MC, curcumin and their combination, it has come out with reasonable clarity about the importance of optimization as the target of any metabolic readjustment. The concept is fully supported by the present finding that there is a total reversal of the shifting trend between MFO, the group responsible for generating free radicals of metabolic need and antioxidant enzymes scavenging the excess free radicals. Under the prevailing metabolic states of the current experimental setup, when the free radicals are necessary scavengers are depressed and the vice-versa. This observation may be regarded as strong support about the authenticity of the experimental setup as the findings of two different parameters are in total harmony with each other.

The lipid peroxide (LPO), which is the oxidative product of different lipids basically, represent the impact of residual oxidative
stress during the act of balancing between the activities of oxidative and free radical scavenging enzyme. The trends of LPO under different conditions of present study indicate 3MC as a potent oxidative stress inducer causing considerable lipid peroxidation. In the present study a single dose of 3MC exposure at the initial period of study is observed to be sufficient enough to maintain persistent lipid peroxidation throughout the period which is supported or rather assisted by concomitant increase in MFO activities and decrease in antioxidant enzymes under 3MC induction. The curcumin modulated effect on peroxidation of lipid is presented by an almost steady state of about 10 percent reduction in lipid peroxidation throughout the period of experiment where metabolic availability of curcumin in maintained through daily administration. The behaviour suggests some form of role for curcumin as a peroxidation arresting agent (Joe and Lokesh, 1994). The observed role of curcumin as an antiperoxidant may be mediated through its capacity to induce the antioxidant enzymes about five fold more than the MFO enzymes which is also minimally induced by a steady state as observed in the present study (Fig.VIII. 1,2). The curcumin is observed to be a very potent free radical scavenging enzyme inducer rather than xenobiotic metabolizing enzyme inducer.

Among the different aspects of curcumin related metabolic changes the role as an antioxidant may be mediated to some extent through induction of the scavenging enzymes. In association with its antioxidant role curcumin is also reported as having some activity in metabolism of xenobiotic substances (Huang et al., 1995), which may be executed through induction of MFO enzymes. In performing the duel function of preventing peroxidation and enhancing xenobiotic metabolism curcumin is observed to maintain a proper balance between the two functions.
The status of LPO under combined effect of 3MC and curcumin is intermediate between the solitary 3MC and curcumin treatment suggesting the early scavenging of free radicals generated by 3MC induced MFO system due to concomitant enhancement of scavenging enzymes by curcumin. The suggested mechanism may play a very important role in reducing or delaying the carcinogenic activity of 3MC in presence of curcumin as observed from the peroxidation trends (Fig.VIII.3) in the present study.

The alpha-fetoprotein(AFP) component representing the biochemical index for the process of carcinogenesis as a cancer marker during the present study is elevated between 400 to 1400 percent with 3MC administration against a maximum elevation of about 200 percent only in a single sample with the curcumin treatment which is within the limit of normal variation suggest an actively ongoing carcinogenesis after 3MC administration which is not present in the control groups. In the phase of experiment with combined 3MC and curcumin treatment the AFP level is increased similarly with that of 3MC treatment in the early part of experiment which is followed by decrease in the later part indicating the delay or arrest in the process of carcinogenesis induced by 3MC under influence of curcumin. The observed trends in the changes of cancer marker also suggest some degree of arrest in the process of carcinogenesis in presence of curcumin (Lin, 2004).

In the present investigation on a consolidated graphical presentation of the total trend of alterations in enzyme activity in comparison to the normal control group it is observed that the 3MC causes the highest alteration initiating with 120 percent which is progressive with duration after the initial single dose (Fig.VIII. 4). Curcumin causes the lowest alteration initiating 40 percent after 30 days.
Fig. VIII.3: Trend of lipid peroxide during experimental period with curcumin, 3-methylcholanthrene and combination of 3-methylcholanthrene and curcumin.
Fig. VIII.4: Resultant trend of different enzymes during experimental period with curcumin, 3-methylcholanthrene and combination of 3-methylcholanthrene and curcumin.

Cuy = -0.0067x' + 1.06x + 14

y = 0.0028x^2 - 0.27x + 124.5
R^2 = 0.8826
R = 0.9394

y = -0.4533x + 119
R^2 = 0.9923
R = 0.996

y = -0.0067x^2 + 1.06x + 14
R^2 = 0.712
R = 0.8438

Days interval

% Deviation
Fig. VIII.5: Metabolic trend during experimental period with curcumin, 3-methylcholanthrene and combination of 3-methylcholanthrene and curcumin.
Fig. VIII. 6: Overall scenario of the study and correlation among the probe parameters.
of regular administration, which increases only by about 5 percent towards the end of the experiment. The combined effect of curcumin and 3MC initiates with the 3MC trend and ends with the curcumin trend suggesting a steady recovery from 3MC induced changes under a persistent curcumin concentration indicated by a very high degree of correlation coefficient of 0.9961.

The present study probing the role of curcumin on some metabolic changes during 3MC induced carcinogenesis is undertaken with MFO enzyme system, three primary antioxidant enzyme system and lipid peroxide as component metabolities. In the foregoing discussion the alteration of these components in the total metabolism of the experimental system is discussed as individual entity with drawing some simultaneous correlation among revealant situations. With an attempt to have a summarized projection of the alterations in the total metabolic scenario under the present experimental setup in the form of the extent of deviation of reorganized metabolism from the normal metabolism, all the fractional observations are graphically presented in a unified form in (Fig.VIII.5). The projection places 3MC as the agent causing highest amount metabolic alteration whereas, curcumin causes the minimal changes. Presence of curcumin with 3MC arrest the severity of metabolic alteration and tend to normalize it with a definite rate as represented by very high degree of regression with \( R=0.9974 \).

In conclusion, the present study provided clear evidence for the suppression of oxidative stress induced by 3MC with accelerated MFO system. From this study curcumin is observed to work in a combination of directly chelating or scavenging effects and by induction of antioxidant enzymes during the process of carcinogenesis.
BIBLIOGRAPHY


