Summary
&
Conclusion
SUMMARY AND CONCLUSIONS

Glutathione S-transferases, a selective adaptation, by the living being in biological evolution can deal with a wide spectrum of exogenous and endogenous reactive molecules in phase II drug detoxicating mechanisms and catalyses the attack on electrophilic reactive intermediates. They are followed by their end product excretion from the cell mainly cytosolic and microsomal proteins distributed in almost all the organs of the living organisms and have several subunits which combine in several combinations to give rise to dimeric protein that can acts on the electrophilic centers of the substrate and reduces their toxicity.

Myriad number of intercalated factors play a pivotal role in maintaining normal physiological conditions. Malfunctioning of any one of these might result in pathophysiological conditions, which is deleterious to life. Cancer- as a diseased state is one such a culmination of more than a factor, where in the normal cells are irreversibly converted to a state where the neoplastic cells loose the potential to control the proliferation. Some of these factors are either toxic electrophiles generated endogenously or xenobiotics to which the biological system is exposed. Cellular system, however, is equipped with detoxification systems, among which GSTs constitutes a primary pathway. Preclinical studies have correlated enhanced metabolism of electrophiles with increased levels of GST isoenzymes with in various tissues. Expression of GSTs in an individual can therefore provide an indicator about the metabolic
potential of their tissues and possible deficiencies in the susceptibility to
dietary or environmental carcinogens. GSTs are over expressed in certain
tumor types, therefore measurement of GST and their subunits in serum or in
pathological specimens can be used as diagnostic markers for certain types of
cancer. Also over expression of GSTs have been implicated for the
development of drug resistance during the course of treatment of cancers.
Therefore, measurement of GSTs can be used to follow the course of disease
and monitor the success of intervention.

Tumor markers are substances that can be detected in higher amounts
than normal in the blood, urine or body tissues of some people with certain
types of cancer. A tumor marker may be produced by the tumor itself, or by the
body in response to a cancer presence. When diagnosing cancer, blood and
pieces of tumor tissues are tested, these tests help to determine the
characteristics of the tumor (aggressiveness, rate of growth and degree of
abnormality).

The human beings are used to expose to various chemical either directly
in factories or indirectly in streets and fields. The chemical entering into the
biological systems are either degraded, or modified and gets involved in
modification of the existing metabolism. The present study was aimed to reveal
the effect of selected chemical toxicants, acrylamide on the rat liver GSTs.

The present study is an attempt to isolate and characterize GSTs
expressed in control and acrylamide treated liver tissues. In order to develop
rapid assay methods to identify individual GST subunits, such as substrate specificity studies with a battery of selected substrates, western blot and dot blot studies. GSTs were purified from rat liver, individual subunits separated on isoelectrofocusing. The salient findings of the present study were summarized below:

1. Rat liver GSTs were purified to electrophoretic homogeneity by GSH-Affinity column chromatography.

2. On SDS-PAGE analysis affinity purified cytosolic liver GSTs were resolved into three bands with relative molecular weights of 28k Da (Yc), 27k Da (Yb) and 25.6 k Da (Ya).

3. These three subunit proteins on isoelectrofocusing were resolved into five basic and seven acidic GSTs isozymes ranging in the PI values from 5-9.9.

4. Substrate specificity analysis showed the expression of different isozymes upon administration of acrylamide. Depending on the dose and time course effect different subunit GSTs were expressed. The conjugation of Yb GST expressions was increased activity with CDNB, BSP, DCNB and EPNP. Yc and Ya GSTs showed more activity with cumene hydroperoxide and H$_2$O$_2$ indicating the increased peroxyl radical concentration in the cell. Depending on the concentration of peroxyl radical either Ya or Yc was expressed more in long term treatment.
5. Polyclonal antibodies were raised in rabbits against affinity purified liver GSTs.

6. In dot blot analysis of acrylamide liver showed the induction of GSTs at 24 mg in short term and at 18 mg in long term acrylamide treatment.

7. Using western blot analysis the polyclonal antibodies with equal concentrations of acrylamide treated liver cytosolic proteins showed induced expression of Yc, Yb and Ya subunits. AC short term treated (24 mg) liver showed induced expression of Yb subunit. At 18 mg liver showed induced expression of Yc and Ya of α class and Yb of μ class in long term treatment.

8. To assess the levels of hepatocellular or blood cell damage in the body the dot blot analysis and ELISA of serum samples was done. In dot blot analysis high immunoprecipitation was observed at 12 mg and 36 mg AC in both short and long term treatments. Using ELISA was also same results observed, high O.D values showed 12 mg and 36 mg AC in both short long term treatments. These studies indicated the damage initiation to erythrocytes and hepatocytes at 12 mg concentration of acrylamide and further determination at 36 mg of AC treatments.

9. To assess the genotoxic effect of acrylamide DNA isolation analyses were carried out using submarine gel electrophoresis. In all the treatments fragmentation of genomic DNA was high.

10. Severity of histological lesions had been observed in multiple doses of short term and long term treatments of acrylamide.
At lethal doses of AC 24 mg in short term and 18 mg in long term the enzyme was (GSTs, GPx I & II) elevated. The quantitation of subunits in both control and treated tissues of liver on immunochemical analysis, and also by enzymatic assays in a dose dependent manner, revealed that Ye, Yb and Ya subunits of the α and μ class were expressed predominantly. The induction studies also revealed that specific subunits are expression at different doses, as confirmed by substrate, transblot and dot blot analyses. The present study suggests that the induction of the above mentioned subunits on acrylamide plays a role in the multi drug resistance mechanism, and that these subunits serves as a markers of neoplasia. Certain tumor markers are simply more accurate than others in their sensitivity to detection of cancer. The more sensitive they are, the earlier it is possible to diagnose. At different concentrations using acrylamide at various conditions as the levels of GSTs were elevated, it may be suggested that GSTs may be used as tumor markers for carcinoma.

In conclusion the acrylamide, a compound which formed during cooking, may cause damage to liver and induces the glutathione S- transferases of liver at the various levels of induction during acrylamide treatments. The immunological, substrate specificity studies and DNA damage studies confirmed the influence of AC on liver and liver GSTs.