Summary & Conclusions
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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, wherein 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 one is exposed. Cellular system, however, are equipped with detoxification systems, among which GSTs constitutes a primary pathway. Pre-clinical studies have correlated enhanced metabolism of electrophiles with increased levels of GST isoenzymes within 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.

Testicular cancer is the most common form among males of age between 15 to 44. After motor vehicle accidents and suicide, cancer is the leading cause of death in this age group, followed by homicide, heart disease, and HIV. Testicular cancer is known as the young man's cancer. Early
detection is the key to survival. Testicular cancer has a very fast onset since the tumors can be very aggressive.

Tumor markers are substances that can be detected in higher than normal amounts 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 tissue are tested, these tests help to determine the characteristics of the tumor (aggressiveness, rate of growth, and degree of abnormality).

The human beings are exposed to various chemicals either directly in factories or indirectly due to pollution. The chemicals entering into the biological systems are either degraded, or modified and gets involved in modification of the existing metabolism. The present study is aimed to reveal the effect of selected chemical toxicants, β-Methylcholanthrene and acrylamide on the rat testicular GSTs.

The present study is an attempt to isolate and characterize GSTs expressed in control and, β-MC and acrylamide treated testicular 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 testis, individual subunits separated on chromatofocusing. The salient findings of the present study are summarized below:

1. Rat testicular GSTs were purified to electrophoretic homogeneity by GSH-Affinity chromatography.
2. SDS-PAGE of affinity purified cytosolic testis GSTs resolved into four bands with relative molecular weights of 27.5(Yc), 26.3(Yb), 26.0(Yβ) and 24.8(Yδ) in kDa.

3. Further separation of affinity purified cytosolic testis GSTs by chromatofocusing were resolved into anionic and cationic GSTs based on their elution order and pH. The anionic GSTs were designated as t₁-t₉. The cationic GSTs were designated as t₁₀-t₉ₐ. The specific activity, total protein and pl (isoelectric point) value to each GST protein was determined. The pl values of rat testis anionic GSTs were ranged from 6.3 to 5.5 whereas cationic GSTs ranged from 9.25 to 8.0. The major isoenzymes which accounted for approximately 60% of the cytosolic testis GSTs were of μ-class, might contribute to the protection of the rat testis from oxidative stress.

4. Polyclonal antibodies were raised in rabbits against affinity purified testis and liver GSTs.

5. In order to understand the role of GSTs on exposure to 3-MC with different intervals and concentrations, GSTs were purified to electrophoretic homogeneity and the results were compared. These studies revealed that α-class GSTs(Yc) and μ-class GSTs (Yb) are expressed predominantly both in short term and long term MC treatments. Further the substrate specificity studies also revealed the elevation of α and μ-class GSTs.

6. In long-term treatment, the decreased specific activity levels were observed for 2 - 4 mg when compared to control ones, the reason for this low activity may be the degradative products of MC due to
metabolic fate, during the course of time may deplete glutathione, and protein-bound sulphydryl (SH) groups, resulting in inhibiting SH-containing enzymes and production of ROS such as superoxide anion, hydrogen peroxide, and hydroxyl radical.

7. Identical to 3-MC with acrylamide at the dosage of 72mg, the electrophoretic studies, substrate specificity studies revealed remarkable induction of α-class and μ-class GSTs in testis. Purification studies revealed appearance of Yp subunit in case of liver reveals that acrylamide may be hepatocarcinogenic.

8. Using Western-blot analysis with class specific polyclonal antibodies (testis and liver) with equal concentrations of both 3-MC & acrylamide treated testis cytosols showed induced expression of Yc and Yb subunits. It was further confirmed by dot-blot analysis taking equal concentrations, using with specific antiserum. It is indicated that Yc of α class, Yb₁ & Yb₂ of μ class were specifically elevated with 3-MC short-term treated (8mg) testis, whereas Yc, Yb₁ and Yc with 12mg testis. Also no significant difference was observed with 1-3 mg (1mg/week) long-term treatment cytosols but difference was observed for 4-6 mg long-term testis cytosolic extracts. Induction of GST enzymes in testis by 3-MC may be due to long-term retention of the carcinogen in the body.

9. Dot blot analysis of acrylamide testis& liver both showed ,besides Yb₁ & Yb₂, revealed that, π form of GST as major subunit..

10. Severity of histological lesions had been observed in multiple doses of short-term (12mg/72hrs) and also in 6mg /6th week MC administered rats making them less fit for better survival. With 72mg dosage of
acrylamide, testis showed aggravated histopathological conditions which was found to be lethal.

11. The substrate specificity, purification, immunological and histopathological studies correlate with the degenerative changes occurred in testicular tissue after treatment with β-MC and acrylamide.

12. Contrast to potent carcinogenic substances like β-MC caused deleterious effects at lower concentration, have been described for acrylamide caused ill effects only in very high concentration.

At lethal doses of 3-MC (8 mg, 12 mg) and acrylamide (72 mg) the concentration of the enzyme was (GSTs, GPx I&II) elevated. The quantitation of subunits in both control and treated tissue of testis on immunoochemical analysis, and also by enzymatic assays in a dose-dependent manner, revealed that Ye Yb1, Yb2, and Yδ subunits of the α,μ and π class were expressed predominantly. The induction studies also revealed that specific subunits are expressed at different doses, as confirmed by substrate and dot blot analysis. The present study suggests that the induction of the above-mentioned subunits on 3-MC treatment 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 3-MC and acrylamide at various conditions as the levels of GSTs were elevated, it may be suggested that GSTs may be used as tumor markers for testicular carcinoma.