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Induction of alpha and Mu-class of rat liver cytosolic glutathione S-transferase upon chronic alcohol administration

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ABSTRACT

The present study reports the effect of alcohol administration on the expression of alpha and mu-class of cytosolic GST in rat liver. One month old Wistar strain rats were divided into two groups i.e., control, and alcohol group. Control group received basal diet and water and alcohol group received basal diet and 10% alcohol for 2 months. Liver was dissected and processed for various biochemical analysis like Western blots, Northern blot, HPLC, IHC and LC-ESI-MS. In alcohol fed group, rGSTM2 protein level was found to be upregulated as evidenced by western blot and HPLC analysis. Increased rGSTA1 and rGSTM2 immunoreactivity in perivenous area was observed compared to controls. In addition, LC-ESI-MS analysis showed an 8 fold increase in rGSTM2 subunit. Differential induction and modification of alpha and mu class rGST subunit and its impaired function may contribute to the progression of alcohol-induced liver damage.

KEYWORDS

Alcohol;
Liver;
Acetaldehyde;
Alcohol dehydrogenase;
Glutathione S-transferase;
Electrospray ionization mass spectroscopy.

INTRODUCTION

The chronic consumption of alcohol is one of the major cause of serious liver diseases and continues to be a major threat to human health. Alcohol is broken down in liver into a number of potentially dangerous by products, such as acetaldehyde and highly reactive free radicals. Despite considerable research accomplishments the biochemical or molecular mechanisms by which ethanol exhibits its hepatotoxic effects remain unknown. The hepatocyte contains three enzyme systems; cytosolic alcohol dehydrogenase (ADH), microsomal ethanol oxidizing system (MEOS), and peroxisomal catalase for oxidation of ethanol to acetaldehyde[1]. Hepatic alcohol dehydrogenase is one of the enzymes responsible for oxidation of 80% ethanol[2]. Long-term intake of alcohol induces alcohol dehydrogenase independent pathways like MEOS and catalase[3]. MEOS involving CYP2E1 metabolizes up to 10% of the ingested alcohol, an enzymatic pathway that plays an important role in drug metabolism, carcinogenesis, steroid and vitamin metabolism[4]. Alcohol is oxidized to acetaldehyde by alcohol dehydrogenase. Acetaldehyde, is toxic and contribute to most of the adverse effects of alcohol which is further metabolized to acetate and water by both cytosolic and mitochondrial aldehyde dehydrogenase (ALDH). However, acetaldehyde reacts with plasma[5], red cells[6] and he-

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patic proteins[7] forming both stable and unstable condensation products, leading to modification in the metabolism of circulating proteins[8], and immunological responses[9]. Previous studies[4,10] have shown that acetaldehyde is associated with increased formation of reactive oxygen species (ROS) and lipid peroxides. The ROS produced may play an important role in the development of oxidative stress in the liver[10,11]. Lipid peroxidation not only perturbs the cellular membrane lipids but also results in the production of aldehyde products such as malondialdehyde (MDA) and 4-hydroxynonenol (4-HNE) that forms shiff's base adducts with proteins[12]. If the enzymatic mechanisms for detoxification of the aldehydes are saturated or decreased, these metabolites can accumulate to toxic levels and potentiate ethanol-induced hepatocellular injury.

Glutathione S-transferases (GSTs; E.C.2.5.1.18) represent the body's important defense mechanism against metabolically generated electrophilic toxins and xenobiotics[13]. Though GSTs are distributed ubiquitously, they have been studied most extensively in mammalian liver, because of their abundance and contribute upto 10% of the extractable protein[14]. GSTs catalyze the nucleophilic attack of glutathione into a variety of potentially genotoxic electrophilic substrates, thereby decreasing their reactivity with cellular macromolecules[15].

A mitochondrial GST, referred to as GST kappa, has also been identified[16]. Cytosolic and mitochondrial GST share some similarities in their three-dimensional fold[17]. The cytosolic GSTs are classified into five classes, alpha, mu, pi, sigma and theta[18]. Among liver detoxifying enzymes, the GSTs play a key role in the protection against oxidative stress, which contributes to the development of liver diseases[19]. Previous studies on the role of GSTs in the protection of the liver from hepatotoxins revealed that a decreased GST activity could result in necrosis[20] and most likely carcinogenesis[21]. Like CYP enzymes, the GSTs can be induced by variety of agents, including phenobarbital, 3-methylcholanthrene, and products of oxidative stress. Increased expression of alpha GSTs in perivenous hepatocytes is found after treatment of animals with 3-methylcholanthrene and phenobarbital[22, 23]. In the present study we have investigated the effect of alcohol administration on the expression of cytosolic GSTs alpha and mu-class, which plays an important role in detoxification and also to regulate other physiological functions that are crucial for normal cellular function and cell viability. Since ADH metabolizes majority of alcohol in cytosol, we intended to study its effect on alpha and mu-class GSTs, which has been reported to be activated by reactive oxygen species[24].

MATERIALS AND METHODS

Reduced Glutathione, epoxy-activated sepharose 6B, 1-chloro-2, 4-dinitrobenzene (CDNB), dithiothreitol (DTT), Nitroblue tetrazolium (NBT), 5-bromo-4-chloro-3-indoyl phosphate (BCIP), Diamino benzidine-Hydrochloric acid (DAB), Triton X-100, Tween-20, Triflouroacetic acid (TFA), phenylmethyl-sulfonyl fluoride (PMSF), Guanidium thiocyanate were obtained from Sigma Chemicals Company (St. Louis, USA). C18 and silica cartridges were obtained from Waters, India. HPLC solvents like acetonitrile, methanol, and water were brought from SRL India Ltd. Nitrocellulose sheet and secondary antibodies were purchased from Bangalore genei, India. All the other chemicals were brought from the local firms and were of high quality.

Animal treatment

One month old Wistar strain rats (100±10g) were used in the present study. Experimental protocols for the use of animals were followed according to Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines and approved by Institutional Animal Ethics Committee (IAEC), University of Hyderabad, Hyderabad, India. The animals were divided into control, and alcohol treated groups. Each group (n=5 animals) had free access to standard commercial rat chow purchased from National Institute of Nutrition, Hyderabad, India. Control group received basal diet and water while the alcohol treated group received basal diet and 10% (v/v) alcohol in water. The caloric percentage of the ingredients of the final regimen (basal diet+alcohol) consumed by the alcohol treated group included 13.8% alcohol, 61.2% carbohydrates, 7.0% fat, and 18.0% protein. Alcohol induced changes in food, fluid consumption, blood alcohol concentration and body weight, have been reported earlier from our laboratory[60]. Blood alcohol

concentration was determined according to the method described earlier[60].

On the last day of experiment (after 2 months of alcohol treatment), animals were sacrificed by cervical dislocation. Liver was dissected and processed for biochemical analysis or stored at -70°C until use for further experiments.

Preparation of cytosolic fraction

The cytosolic fraction was prepared from control, isocaloric and alcohol-fed rat liver as described by Kim et al.[25]. Briefly, tissues were homogenized separately in 10mM Media-I, pH 7.2 (10mM K_2HPO_4, 1mM EDTA, 2mM DTT and 250mM Sucrose) at 4°C using potter Elvejahm homogenizer. The homogenate was then passed through layers of cheesecloth and the filtrate was centrifuged at 10,000×g for 30 minutes. The resulting supernatant was filtered through glass wool to remove the floating lipid material and the filtrate centrifuged at 105,000x g for one hour in Hitachi ultracentrifuge with p50AT2 rotor and the resulting supernatant was referred to as crude cytosolic fraction.

GST assay

GST activity was measured essentially as described by Habig et al.[26]., using 1, chloro 2,4-dinitrobenzene as substrate. The standard assay mixture contained 1mM CDNB (1,chloro 2,4-dinitrobenzene), 1mM reduced glutathione (GSH) and 100mM potassium phosphate buffer (pH 6.5) in a volume of 1 ml. The optical density was read at 340nm.

Purification of liver cytosolic GST

Cytosolic samples were loaded on GSH-Sepharose 6B affinity column which was pre-equilibrated with buffer A (10mM K_2HPO_4, 1mM EDTA, 2mM DTT). The column was washed with buffer A containing 150mM of KCl, until no protein (estimated by A_{280}) could be detected in the effluent. The adsorbed protein was then eluted with buffer A containing 5mM GSH and collected as 1 ml fractions. The fractions exhibiting highest GST activity were pooled and stored at -70°C till further use. GST purity was checked by SDS-PAGE as described by[61]. Protein concentration was estimated by the method of Lowry et al.[27].

Western blot analysis

Proteins from control and alcohol-fed rats (50µg) were transferred from 12.5% Polyacrylamide gels to nitrocellulose membranes. Protein transfer was performed at 45 mA for two and a half hours in a semi-dry electroblotting apparatus[28]. The non specific sites were blocked with 5% non-fatty milk powder in TBS (10mM Tris pH 8.0, 150mM sodium chloride) for 1hr at room temperature, and then incubated with primary antibody for 1hr at room temperature. After extensive washing with TBST (TBS containing 5% Tween-20) blots were incubated with goat anti-rabbit antibody conjugated to alkaline phosphatase for 1 hr. After washing, blots were developed in alkaline phosphatase buffer (10mM Tris, 5mM $MgCl_2$ and 100mM NaCl, pH 9.5) containing 0.033% NBT and 0.0165% BCIP. The membrane was dried and densitometric analysis was performed using NIH Image software (Scion Image).

Northern blot analysis

Total RNA was isolated from control and alcohol-fed rats as described by Chomczynski and Sacchi[29]. 20 µg of total RNA was separated on 1.2% formaldehyde agarose gel and transferred to a nylon membrane. Filters were prehybridized and then hybridized with (α-^{32}P) dCTP-labeled GST cDNA probe. After hybridization, filter was washed, dried and autoradiographed at -80°C using Fuji RX-NIF x-ray film. Hybridization signals were quantified using Bio-Rad image analysis densitometer. The signals were corrected for unequal loading of the cellular RNA by normalization to 28sRNA content.

Immunohistochemistry

After transcardial perfusion of animals using 4% paraformaldehyde, liver tissue was post fixed in 4% paraformaldehyde. Briefly, deparaffinised sections of 5µm were treated with 3% hydrogen peroxide in deionized water for 30 min and washed in TBST. The sections were blocked with 1% of goat serum in TBS for one hour at room temperature. The sections were incubated at 4°C for 12-14 hrs with rabbit antibody against rGSTM1 and M2 using incubation chamber. The sections were rinsed with TBST followed by incubation with anti-rabbit secondary antibody conjugated to HRP for 1hr at room temperature. Slides were washed and

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developed using freshly prepared 0.03% (w/v) DAB (3-3' Diaminobenzidine) and 0.03% H_2O_2 (v/v) as substrates and developed until the desired brown reaction was monitored under the microscope. The reaction was terminated by gently rinsing with distilled water. The slides were counterstained with Mayer's haematoxylin for 2 min and mounted in Dpx. Control reactions were performed without specific antiserum and with non-immune rabbit serum.

High pressure liquid chromatography (HPLC) analysis

High performance liquid chromatographic analysis[30] of affinity purified GST[31] was carried out on a 10cm×0.8cm Waters μ Bondapak C18 reverse-phase column (Waters Corporation, Milford, MA, USA) (pore size 5μ) in a Z module with Waters systems employing gradient elution. The solvents were 35% (v/v) acetonitrile with 0.1% (v/v) trifluoroacetic acid (Solvent A) and 85%(v/v) acetonitrile with 0.1% trifluoroacetic acid (Solvent B). 50 μg of affinity purified cytosolic GST was injected at 35% v/v solvent A and eluted with a linear gradient run from 35% to 55% solvent B over 70 min, with a flow rate of 1ml/min. Polypeptides were detected at 214nm. The separation of subunits by HPLC was quantified from peak area expressed as A214×ml, as obtained from the recorder. In order to convert peak area into protein content, the ε214 for each subunit obtained by multiplying its ε280, calculated from its known tyrosine and tryptophan content, by the ratio of A214 to A280 obtained from absorption spectrum. Individual peaks were characterized on the basis of their anodal mobility on SDS-PAGE.

Molecular-mass determination of rGSTM1/M2 subunit by liquid chromatography-electrospray ionization mass spectroscopy (LC-ESI-MS)

Mass spectral analysis of rGSTM1 and M2 subunit was performed by on-line HPLC connected to the mass spectrometer. The instrument was set in the positive mode and nitrogen gas was used as the nebulizer gas with a capillary voltage of ~3.8KV. Scanning was done in multi channel analyzer mode from m/z 800 to m/z 2000, at a cone voltage of 20V. Data was summed according to the total ion current profile and processed by masslynx ver. 3.2. The raw data was subtracted for background noise smoothed and centrioded to calculate the mass of the sample.

Statistical analysis

Statistical analysis was performed using paired t-test, employing the sigma plot software. A value of $p<0.01$ was considered significant.

RESULTS

Western blot analysis was performed using affinity-purified antibody raised against individual GST isoforms

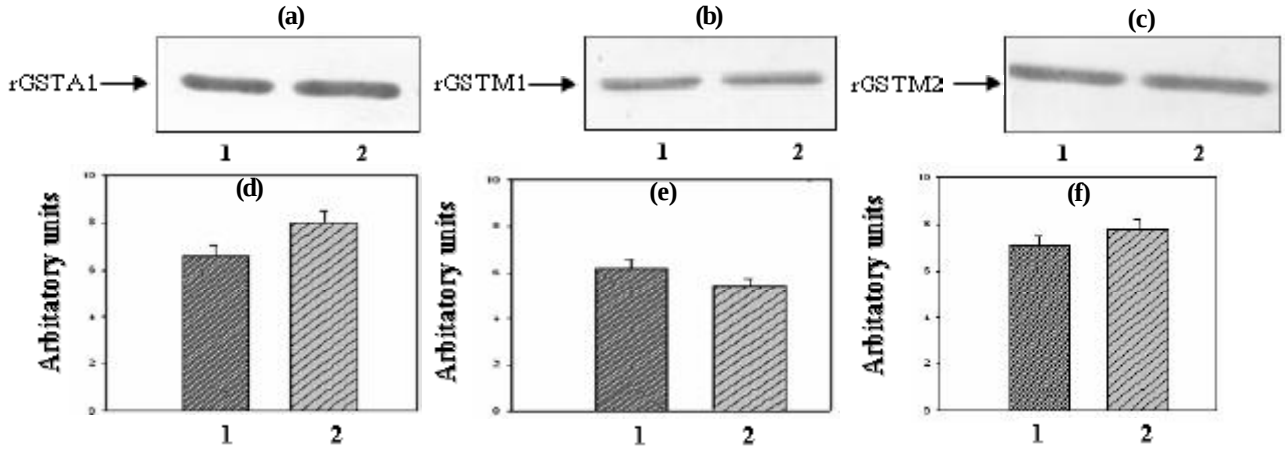

Figure1: Effect of alcohol on hepatic rGSTA1, rGSTM1/M2 subunit. Affinity purified cGST proteins from control, and alcohol-fed rats were subjected to immunoblot analysis with anti- rGSTA1 (a), rGSTM1 (b) and rGSTM2 (c) antibody as described in materials and methods. 'a' ,'b' and 'c' represents immunoblot with 50μg of protein loaded per lane. 'd' , 'e' and 'f' represent densitometric analysis for 'a','b' and 'c' respectively. Each data point represents the mean from five analysis. Student's t-test statistical analysis was used. A value of p<0.01 was considered significant.

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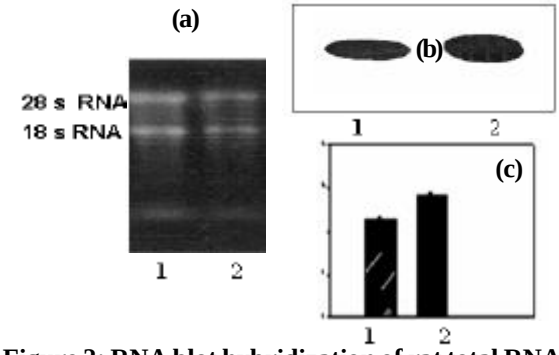


Figure 2: RNA blot hybridization of rat total RNA with rGSTM1/M2 cDNA probes. 20μg of total RNA was loaded from control and alcohol-fed rat liver in each lane.

(a). Blots were hybridized with ^{32}P-labelled cDNA probe corresponding to rGSTM1/M2 mRNA (b) and rGSTA1 (c). The blots are represented along with the densitometric analysis. Each data point represents the mean from five analysis. Student's t-test statistical analysis was used. A value of $p<0.001$ was considered significant

(rGSTA1, rGST M1 and rGST M2). In the current study, alcohol was shown to increase rGSTA1 and rGSTM2 protein levels (figures 1a, 1b and 1c).

After electrophoresis and transfer of total RNA (20μg) (figure 2b) from control and alcohol group, the levels of mRNA for α-class and mu class of GST was detected by random primer labeled cDNA probe. mRNA for mu-class GST revealed an increase of 113.27% of control upon alcohol administration.

Immunohistochemistry of liver sections showed a uniform distribution of rGSTA1, rGSTM1 and M2 in control (figures 3A, 3C and 3E) and enhanced immunoreactivity in perivenous area (zone III) for A1 and M2 subunit in alcohol-fed rat (figures 3B and 3F), in contrast, M1 subunit immunoreactivity was found to be

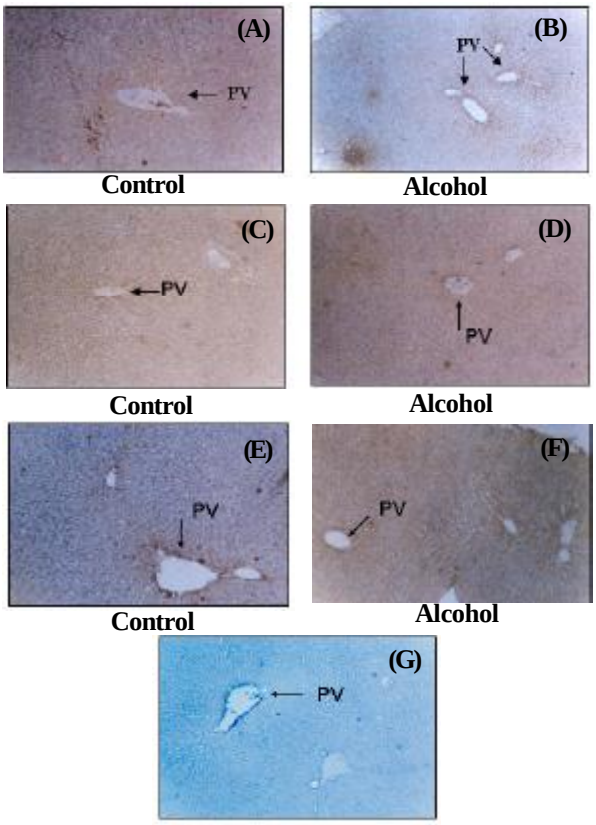
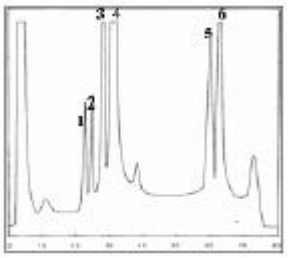


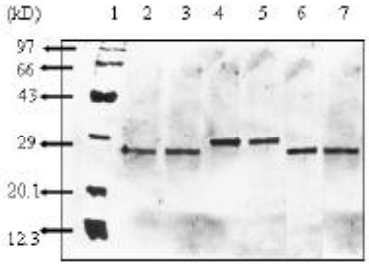
Figure 3: Immunohistochemical localization of rGSTA1, rGSTM1 and M2 subunits in control, and alcohol-fed rat liver. rGSTA1(AandB), rGSTM1 (CandD) and rGSTM2 (EandF) enzyme was demonstrated in both the control and alcohol-fed animals. All the GST subunits shows a uniform distribution of GST enzyme in rat control liver. But, in alcohol fed rats the staining intensities for all the GST subunits was found to be markedly higher in perivenous area (PV). Sections treated with preimmune serum were considered as negative control (G). Original magnifications, ×250

(A) represent RP-HPLC analysis of GSH- affinity purified cytosolic GST from normal rat liver (50 μg)



Column: water μ-bondapak C18 (0.39-30 cm). Solvent A: 0.1% TFA in 35% Acetonitrile, Solvent B: 0.1% TFA in 85% Acetonitrile, Gradient: 0 min B. con. 1%, 20 min B.con. 30%, 30 min B.conc.30% , 55 min B conc. 40%, 60 min B.conc. 60%,65 min B.conc 70%,70 min B.conc. 1%, 75 min stop

(B) represent's SDS -PAGE analysis of fractions collected from RP-HPLC column.



Lane 1: mol.Wt. Marker
Lane 2: Peak 1
Lane 3: Peak 2
Lane 4: Peak 3
Lane 5: Peak 4
Lane 6: Peak 5
Lane 7: Peak 6

Order of elution of cytosolic GST on C-18 column: μ-class (Yb1), μ-class (Yb2), α-class (Yc1), α-class (Yc2), α-class (Ya1), α-class (Ya2);

Figure 4: Identification of individual cytosolic GST isoform separated on C-18 column.

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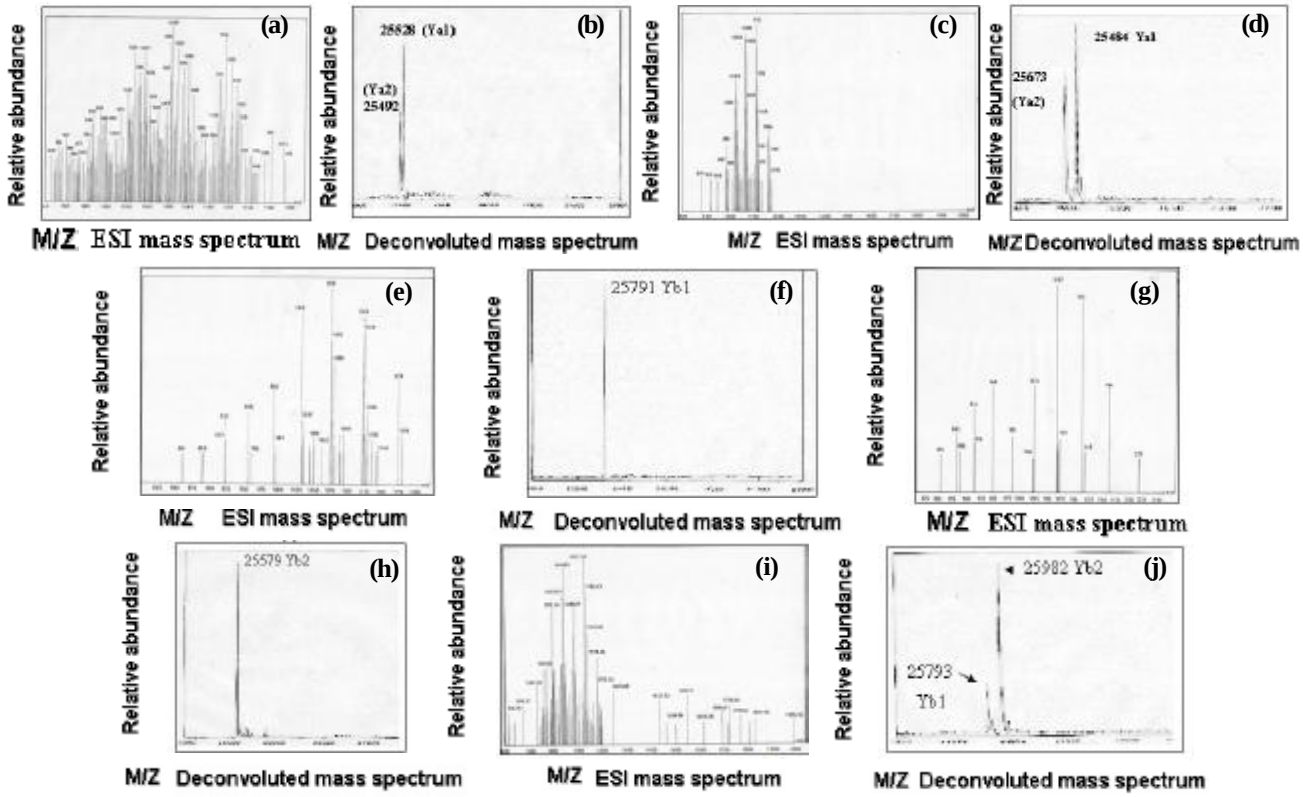

Figure 5: LC-ESI-MS analysis of cGST. Individual peaks obtained from RP-HPCL were subjected to ESI-MS analysis from control and alcohol fed rat liver. (a) represents ESI mass spectra, (b) represents deconvoluted mass spectrum.
'A' represents ESI mass spectra and deconvoluted mass spectrum in control and alcohol fed rats for rGSTA1 and A2 protein respectively; 'B' represents ESI mass spectra and deconvoluted mass spectrum in control and alcohol fed rats for rGSTM1 and M2 protein respectively.

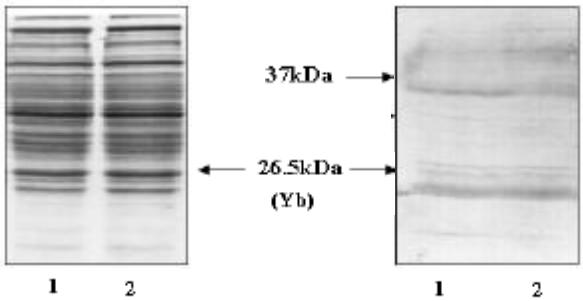

Figure 6: Total cytosolic proteins from control and alcohol-fed rats were subjected to immunoblot analysis with serum collected from control and alcohol-fed rat as described in materials and methods: (a) represents SDS-PAGE with 50μg of protein loaded per lane, (b) represent immunoblot

TABLE 1: Relative abundance of cytosotic glutathione-s-transferase subunits in control, alcohol and alcohol withdrawal rat livers

S. no.	Sub units/class	Control		Alcohol	
		Retension time(min)	Abundance (μg)	Retension time(min)	Abundance (μg)
1	μ1 (Yb1)	21.033	1.105	20.817	0.44
2	μ1 (Yb2)	23.03	1.095	21.78	8.8

decreased (figures 3D). Figure 3G shows liver sections probed with preimmune sera which did not show any immunoreactivity (negative control).

The affinity purified cGST from rat liver were analyzed and quantified by RP-HPLC. A typical chromatogram is shown in figure 4. GSTs in alcohol fed rats revealed an 8 fold increase in rGSTM2 subunit (TABLE 1). Mass spectra analysis of control affinity purified HPLC peaks were 25791 for rGSTM1, 25579 for rGSTM2 (figure 5 f and h), 25528 for rGSTA1 and 25492 for rGSTA2 subunits (figure 5b). Alcohol fed affinity purified GSTs showed Molecular masses 25793 for rGSTM1, 25982 for rGSTM2 (figure 5j) and 25484 for rGSTA1 (figure 5d).

The generation of autoantibodies was studied by probing cytosolic fraction with antiserum collected from alcohol-fed rats (figure 6a). A positive immunoreactivity was observed for alpha and mu-class subunits, which further confirms the generation of autoantibodies against cGST upon alcohol administration in addition to other

proteins (figure 6b).

DISCUSSION

GSTs represent one of the major cellular defense mechanisms against electrophilic xenobiotics and their metabolites. Since it plays an important role in detoxification and other physiological functions, we studied the effect of alcohol administration on GSTs of cytosol, where majority of alcohol is metabolized.

Western blot analysis on affinity-purified antibody raised against individual GST isoforms (rGSTA1 and rGSTM2) showed preferential increase due to alcohol administration. It has been shown that mu-class GSTs are sensitive to ROS[24]. The increase in both alpha and mu class GSTs may play a crucial role in protecting the liver cells against toxic effect of alcohol and its metabolite. It is likely that the cellular responses might really involve interplay among these specific classes and isoforms, and the alterations in GST profile may drive cell towards dysfunctional apoptosis.

It has been reported that cadmium treatment increased alpha-class glutathione-*S*-transferase proteins by about 25% in rat liver cytosol[32]. It is unlikely that the stimulatory effect was due to the high level of peroxides caused by lipid peroxidation, since Vitamin E administration strongly reduced the peroxides level, but did not affect the GST activity[32]. It has been shown that altered levels of alpha-GSTs are a very sensitive marker in assessing cellular damage induced by malnutrition[33]. In alveolar epithelial cells, cadmium was found to increase the activity and expression of alpha- and pi-GST classes with concomitant ROS production[34]. It has been reported that in liver cytosol GST activity increases after acute cadmium intoxication of rats[35]. At the same time, higher levels of liver TBARS formation have been observed. It has been hypothesized that alpha-class GSTs are those mainly involved in this process.

Studies reported that there was a significant decrease in cytosolic GST due to ethanol exposure in primary culture of mouse hepatocytes[36]. Mu and pi- class GSTs was decreased by 53% and 13% respectively. These changes were completely or partially reversed by either Vit-E or alcohol dehydrogenase (ADH) inhibitor like 4-methylpyrazole. Brind's et al.[37]. have studied the genetic susceptibility of alcoholic liver diseases and significant association of GST polymorphism with particular reference to GSTM1, M3, P1, T1 and A1 by PCR. The study fails to demonstrate beyond doubt a link between the two.

RNA blot hybridization analysis revealed a significant increase in the levels of mu-class rGST mRNAs and parallels the increase in net GST protein production. Increased mu-class rGST production could be the result of increased transcription or stabilization of mRNA. Previous studies have demonstrated that chronic ethanol ingestion increases hepatic mRNA level and protein synthesis. Iron overloading caused a liver mRNA increase in mouse, with consequent higher protein levels of GSTA1, GSTA4 and GSTM1. The GSTA4 induction was related to ROS production by iron exposure[38]. In addition Baraona and co-workers[39] found that the absolute synthesis of hepatic protein was increased in *in vivo* in ethanol-fed rats.

The isozyme is an alpha class GST that demonstrates substrate specificity which may be particularly important for cellular defense against oxidative stress[40]. GSTA4-4 utilizes 4-HNE as its preferred substrate, conjugating it to GSH with high affinity[41,42]. The ability of GSTA4-4 to metabolize electrophiles generated during oxidative stress suggests that this isozyme may function as a major defense mechanism against the liver injury induced by CCl4. It is reported that in mGSTA4-4 knockout mice CCl4- mediated hepatotoxicity is exacerbated by the initiation of rapid lipid peroxidation leading to an increase in intracellular 4-HNE concentration and also it plays a significant protective role only during the early stages of this toxic insult[43].

Extensive deletions in GSTM1 and GSTT1 result in complete loss of enzyme function, which possibly influence colorectal cancer susceptibility[44]. Therefore, a large number of studies have been performed to assess whether GSTM1-deficiency or other GST polymorphisms are associated with colorectal cancer susceptibility[45,46]. The results were heterogeneous and failed to demonstrate any link.

Immunohistochemical studies using polyclonal antibody in hepatic lobule showed increased alpha rGSTA1 and mu rGSTM2 immunoreactivity in perivenous area in alcohol fed liver. The enhanced intensity in zone III could be due to ethanol-related in-

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crease in oxidative stress induced by the presence of high activity of alcohol dehydrogenase and CYP450 in this area[47]. It was reported that the ethanol fed animals when subjected to experimental hypoxia, the cellular injury was increased in the perivenous region[48,49], suggesting that ethanol consumption and oxygen deprivation showed additive effects in this region. Taken together, we believe that the product of ethanol metabolism might account for the observed induction of rGST subunit.

The peroxidase activity associated with the GSTs is referred to as NonSe-GPX activity, which represents one of the important antioxidant mechanisms that exist in cells for protection against hydroperoxides[50]. In humans, the NonSe-GPX activity of GSTs towards lipid hydroperoxides is mainly associated with the Alpha class of GSTs[51]. The bulk of the alpha GST activity is exhibited by the cytosolic isoforms like GSTA1, A2, A3, and A4[51,52]. The microsomal GSTA1-1 showed significant NonSe-GPX activity towards physiologically relevant fatty acid hydroperoxides, the biogenesis of which is directly linked to oxidative stress[53]. Therefore, the conjugation and peroxidase activities associated with GSTs towards a wide variety of electrophilic compounds may be considered as an integral part of the cellular antioxidant defense system and it mitigates the toxic effects of ROS.

Further, HPLC analysis of affinity purified GST showed an 8 fold increase in rGSTM2 subunit. The mass difference between native peptide and adduct molecules is consistent with a schiff base between an amine and acetaldehyde. Mass spectra show no masses consistent with fragments from either intra or interpeptide cross-linking. In addition, the modified protein appeared as a single peak on C18 RP column, where the cross-linking of some of the molecules would change chromatographic behavior sufficiently to generate an additional peak.

It is suggested that the alpha class GSTs play an important role in regulation of the intracellular concentrations of the products of lipid peroxidation that may be involved in the signaling mechanisms of apoptosis[51]. These results show that the transfection of K562 cells with *hGSTA2* attenuates H_2O_2-induced apoptosis by suppressing SAPK/JNK activation and caspase 3-mediated PARP cleavage. The physiological significance of the GPx activities of hGSTA1-1 and hGSTA2-2 has not been systematically investigated. A subgroup of the Alpha class GSTs having substrate preference for 4-HNE is also present in mammals including human cells[54,55]. It demonstrates that hGSTA1-1 and hGSTA2-2 can reduce PL-OOH in the biological membranes *in situ* and that the overexpression of hGSTA2-2 protects K562 cells from H_2O_2-induced LPO and cytotoxicity.

Our laboratory has demonstrated earlier that the toxic products generated endogenously from alcohol metabolism (HNE, MDA, and acetaldehyde) are accumulated in perivenous area[56]. In addition the inhibition of GSTs function as reported in this study might increases the susceptibility of this region for cellular dysfunction. The level of GST expression is considered to be an important factor to protect organs against the deleterious effect of toxicants. Most GST inducers include phenols, N-heterocyclic compounds and dithiothiones. Most of these compounds are used as anticancer drugs. This effect may represent an important cellular mechanism against oxidative stress[57,58]. Xenobiotic GST inducers such as phenobarbital or benzonaphthoflavone have been reported to increase the class alpha GST in the cytosol of rat liver[59]. Heavy metal intoxication has been shown to have some influence over GST expression. Studies on the effect of iron overload on GST expression in mouse liver and kidney have been reported[38]. It was found that the levels of GSTA1, A4 and M1 increased in liver while the expression of GSTA1 and M1 is reduced in kidney, where only the GSTA4 level increased. The GSTs increase the efficiency of glutathione-dependent detoxification of electrophilic xenobiotics and the by products of oxidative stress that are critical to cellular homeostasis[51]. Alcohol consumption showed an increase in protein and mRNA expression. The increased expression of rGSTM2 subunit and the formation of acetaldehyde adduct indicate the modification of rGSTM2 subunit. Thus, its impaired function in alcohol administered rats may contribute to the progression of alcohol induced liver damage.

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Abbreviations:

MEOS - Microsomal ethanol oxidizing system; ADH - Alcohol dehydrogenase; ROS - Reactive oxygen; ALDH - Aldehyde dehydrogenase; MDA -Malondialdehyde; 4HNE -4-hydroxynonenol; GSTs -Glutathione S-tranferases; CYP -Cytochrome P

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