RESULTS & DISCUSSION
4. RESULTS AND DISCUSSION

Fulminant hepatic failure (FHF) is a challenging syndrome in clinical medicine and is a devastating illness that has a high mortality rate (Aw & Dhawan 2002). All patients should be managed in an intensive care setting pending transfer to the liver transplantation center. The postoperative course of the sick patients, and the fact that liver transplant should take place before severe irreversible brain damage has ensued; make the transplantation for FHF a very challenging field (Bernstein & Tripodi, 1998). A better understanding of mechanisms responsible for liver cell death and multiorgan failure, and the development of strategies to enhance liver regeneration, may allow a more targeted approach to therapy. Treatment with effective hepatoprotective and cytotrophic drugs may allow the native liver to regenerate (Lesnikov et al., 2004). The major abnormalities noticed in FHF are oxidative free radical damage, loss of plasma membrane integrity and hyperlipidaemia (Day et al., 2004). It is appropriate thus to use a biological molecule devoid of adverse side effects, as a therapeutic to effectively counteract the above mentioned aberrations. Also it could be of great significance if such a molecule was endogenous in nature with several roles to play in the living cell. Taurine, as discussed in this chapter has shown a protective effect by amending the D-GalN-induced derangements in the liver.

4.1 Liver diagnostic enzymes

A significant rise occurred in the levels of liver specific marker enzymes namely, ALT, AST, LDH, ALP, ACP and GGT in the plasma of Group III galactosamine-induced hepatitis rats in comparison with that of Group I control rats (Fig 4.1.1 and 4.1.2). This is in agreement with earlier studies (Anandan et al., 1999) where it was reported that parenchymal damage to the hepatocyte resulted in spillage of liver enzymes into the
blood. These studies support the view that a single acute dose of D-GalN produces, within 24 hours, foci of hepatocellular necrosis throughout the liver.

Serum ALT and AST are sensitive indicators of liver injury. The extent of hepatic damage is assessed by the serum level of enzymes released from cytoplasm and mitochondria (Daba & Abdel-Rahman, 1998). The precise levels of these enzymes correlate well with the extent of liver damage or the prognosis. The highest levels of liver specific enzymes are found with disorders that cause extensive hepatic necrosis as in acute viral hepatitis or due to pronounced liver damage inflicted by toxins like D-GalN or acetaminophen (Thapa & Walia, 2000). Of all the macromolecules that leak from damaged tissues, enzymes because of their tissue specificity and catalytic activity are the best markers of damage (Ebenezar et al., 2003). The release of cellular enzymes reflects induction of intracellular stress, accompanied by non-specific alterations in the structural and functional characteristics of liver cell membranes altering their integrity and permeability. The depletion of nucleotides due to D-GalN ultimately impairs the synthesis of protein and glycoprotein of cellular membranes which leads to enzyme leakage from the cells (Keppler et al., 1970; Abdul-Hussain & Mehendale, 1991). This study revealed a significant increase in the activities of serum ALT and AST with the exposure to D-GalN could be inhibited by the oral administration of taurine, demonstrating a hepatoprotective effect for compound. The effect on serum AST also suggests that mitochondria are protected, because 80% of AST is released from mitochondria (Daba & Abdel-Rahman, 1998). The protective effect of taurine on both hepatocytes and their mitochondria was further demonstrated by ultrastructural examination.

In the present study, pre-treatment of rats with taurine at a dose of 100mg/kg body weight in Group IV resulted in significant (p<0.001) prevention of D-GalN-induced rise in the levels of diagnostic marker enzymes when compared to Group III D-GalN-intoxicated rats. The attenuation of the liver specific marker enzymes in blood by taurine is a clear indication of its
cytoprotective and membrane stabilizing effect. Taurine has a role in a number of crucial processes, including calcium ion flux and membrane stabilization (Kendler, 1989). The suggestion that taurine is a membrane stabilizer comes from the evidence that it is needed to maintain the structural integrity of photoreceptor membranes in vivo (Pasantes-Morales & Cruz, 1984; Pasantes-Morales et al., 1983) and from its ability to counteract damage caused by external agents in a variety of membrane preparations (Wright et al., 1986, Kramer et al., 1981, Pasantes-Morales et al., 1981, Pasantes-Morales et al., 1984b; Huxtable & Bressler 1973).

4.2 Histopathology

Histopathological examination had been carried out in the liver tissue of control and experimental groups of rats to confirm the cytoprotective nature of taurine against D-GalN-induced hepatic failure. Light microscopy of the liver tissue sections of Group I control rats showed normal liver architecture namely hepatic chords, centri-lobular vein and sinusoid capillaries with regular aspect (Plate 4.2.1). Histopathological examination of livers from the D-GalN-treated rats demonstrated the loss of parenchymal liver cells from both central venule and periportal areas (Plate 4.2.3). Other changes include severe and diffuse areas of hepatitis, especially in the periportal areas, focal necrosis and inflammatory infiltration, fatty change, sinusoidal distension and an increased number of Kupffer cells. These observed D-GalN-mediated hepatotoxic effects were similar to those previously reported (Ito et al., 2008; Kasai et al., 2001).

In contrast, rats administered with 100 mg/kg taurine prior to D-GalN administration were completely protected against D-GalN -induced aberrations in the hepatic structure. Taurine reversed, to a large extent, the hepatic lesions produced by D-GalN, as is obvious from the absence of cellular necrosis, fatty accumulation, Kupffer cells and lymphocytes
infiltration around the portal area (Plate 4.2.4). Only spotty necrosis of hepatocytes and a slight inflammatory reaction was found in mice administered with taurine which is a clear indication of the cytoprotective effect of taurine. The histological examinations of the liver tissue of normal rats receiving taurine (Group II) alone did not show any significant changes when compared with that of normal control rats, showing that it dose not per se have any adverse effects (Plate 4.2.2). Similar results have been reported wherein taurine was shown to protect liver architecture from oxidative damage (Yalçınkaya et al., 2009).

4.3 Serum bilirubin and prothrombin time

Two of the several parameters by which hepatotoxicity induced by D-GalN is usually judged are elevated serum total bilirubin and prothrombin time. Serum total bilirubin was significantly (p<0.001) increased (Fig 4.3.1) over those in normal Group I rats after injection of D-GalN in Group III rats. This rise in serum bilirubin correlated with the onset of massive hepatic lesions. Similar results are reported by other researchers (Vimal & Devaki, 2004). Increase in bilirubin levels reflects hepatocyte and bile duct injury and the rise has shown to be proportional to the extent of liver and bile duct damage (Mitra et al., 2000) D-GalN impairs hepatic function and one of its manifestations is the marked abnormalities in bilirubin metabolism which results in jaundice. Liver paraenchymal damage causes increase of bilirubin in circulation as conjugation with glucuronic acid an enzymatic reaction catalyzed by a liver enzyme is affected (Peters & Jansen 1986). Administration of rats with taurine in Group IV significantly (p<0.001) reduced (Fig 4.3.1) the levels of serum total bilirubin when compared to rats of Group III. In studies examining the role of taurine in ameliorating hyperbilirubinemia, it was demonstrated that taurine improves excretion of bile, blood flow, and augments the functions of hepatocytes (Miyata et al., 2006; Guertin et al., 1991).
Prothrombin time is a measure of time required for blood to clot and is a parameter for assessing hepatic protein synthesis, since clotting depends on blood clotting factors synthesized in the liver (Wada et al., 2008). A notable change in blood coagulation was observed as indicated by a significant (p<0.001) prolongation (Fig 4.3.2) of prothrombin time in D-GalN intoxicated mice Group III rats when compared to normal Group I rats. This is in agreement with similar reports which show that D-GalN intoxication enhances prothrombin time by impairing hepatic protein synthesis (Wang & Li 2006; Arvelo et al., 2002). Administration of taurine in Group IV rats significantly shortened the prothrombin time by 18-20% when compared to D-GalN-intoxicated Group III rats.

The results of present study demonstrated that D-GalN caused a series of events including elevation of serum aminotransferase, jaundice, and dysfunction of blood coagulation and necrosis of hepatocytes that may ultimately lead to lethal shock. Such biochemical and pathological changes clearly resemble acute hepatic failure in human. Serum total bilirubin and prothrombin time are considered important indicators that reflect the prognosis of acute hepatic failure (Batra & Acharya, 2003). In the current study, administration with taurine offered significant protection from the lethal shock of mice and ameliorated liver function by lowering total bilirubin levels and reversing increases in prothrombin time. The levels of these parameters were normalized through the cytoprotective effect of taurine as indicated by the alleviation of hepatic injury such as inflammation and necrosis induced by D-GalN.

4.4 Plasma urea & arginase and liver arginase

Arginase is a urea cycle enzyme that is specific to liver mitochondria. There was a significant (p<0.001) rise (Fig 4.4.1) in the levels of arginase in the serum of Group III D-GalN-induced hepatic failure rats when compared to Group I normal rats. This finding is in agreement with several other reports (Murayama et al., 2008) that show that liver injury in
toxicant-induced acute hepatotoxicities, leakage of a marker into the circulation is influenced by the character of the marker, rather than its localization and thus, arginase a mitochondrial enzyme is a marker that aids in the detection of hepatotoxicities. Earlier mitochondria-derived markers have been believed to be less sensitive in the detection of hepatic injuries than cytosol-derived markers due to prior reports of slow release of mitochondrial enzymes such as glutamate dehydrogenase in acute hepatic injury induced by CCl₄ (Murayama et al., 2009). Recently, mitochondrial dysfunction has been believed to be a cause, rather than a consequence, of cell injury in some forms of cell death (Fiskum, 2000), which might suggest that some mitochondria are destroyed before plasma membrane injures; therefore, some mitochondrial markers might be as sensitive as cytosolic markers. D-GalN through inhibition of protein synthesis causes defective membrane glycoprotein assembly which results in impairment of mitochondrial structural integrity and function. Also by induction of oxidative stress to which mitochondria are specifically sensitive, D-GalN causes massive liver necrosis and triggers the signaling pathways that lead to apoptosis in mitochondria spilling the enzymes like arginase into the bloodstream (Balkan et al., 2001).

In the present study administration with taurine in Group IV rats has restored the levels of arginase (fig 4.4.1) to reasonably normal levels when compared to Group III rats. These results agree with similar published reports. Taurine is reported to preserve cell membrane integrity and liver ultrastructure (Ates et al., 2006), which possibly prevent the D-GalN-mediated injury to mitochondria and subsequent leakage of mitochondrial enzymes into the extracellular fluid (Tasic et al., 2008). Also the potent antioxidant effect of taurine is believed to have a protective effect on the mitochondria preventing oxidative damage (Refik Mas et al., 2004; Messina & Dawson 2000).

Deamination of aminoacids produces ammonia which is a toxic compound. Liver contains a system of enzymes catalyzing a series of reactions, to detoxify ammonia by its conversion
to urea, referred to as urea cycle. Loss of liver function drastically reduces the activity of urea cycle enzymes causing an accumulation of ammonia that creates serious complications (Dams et al., 2008). In the present study, the activity of arginase in liver was significantly (p<0.001) reduced (Fig 4.4.2) in rats treated with D-GalN (Group III) when compared to Group I normal rats. This is in agreement with results reported elsewhere signifying the fact that urea cycle is depressed in liver failure causing an accumulation of toxic compounds like ammonia. Deficiencies of individual urea cycle enzymes have been implicated in liver diseases (Mehler 1993). Earlier studies by (Anandan et al., 1999) showed decreased activities of urea cycle enzymes during the induction of D-GalN hepatitis in rats. In accordance with this reported study, the present results elicited a significant decrease in the activity of liver arginase. The presence of excess ammonia along with other toxic compounds in blood as a result of decreased detoxification by liver leads to encephalopathy (Elgouhari & O'Shea 2009; Jiang et al., 2009), a common complication in fulminant hepatic failure.

Administration of rats in Group IV with taurine has resulted in significantly (p<0.001) elevated (Fig 4.4.2) levels of arginase in liver when compared to Group III D-GalN-intoxicated rats. These results indicate that taurine by virtue of its membrane stabilizing property and antioxidant property had effectively prevented the D-GalN-induced aberrations in liver structural integrity and preserved liver function. Similar results were reported by other researchers substantiating the observations of this study. The roles of taurine thus far elucidated include membrane stabilization, osmoregulation, bile salt formation, growth regulation, calcium homeostasis, and apoptosis modulation (Redmond et al., 1998), all of which contribute to a cytoprotective effect against a variety of mechanisms of cell damage (Schaffer et al., 2003).

Because urea is synthesized by the liver and there is loss of activity of liver enzymes in severe liver failure, there is reduction in the levels of urea in the blood (Brzóska et al., 2003).
In the present study urea levels were significantly reduced (Fig 4.4.3) in the plasma of rats of D-GalN-intoxicated Group III rats when compared to normal Group I rats. D-GalN intoxication causes lowered urea levels in plasma due to decreased activity of urea cycle enzymes as seen by decreased activity of arginase in this study. Arginase is the enzyme that cleaves arginine to give urea and ornithine.

Administration of rats with taurine in Group IV has resulted in elevation (Fig 4.4.3) of levels of plasma urea when compared to Group III D-GalN-intoxicated rats. Taurine improves the performance of urea cycle as it restores the activities of enzymes by preserving the ultrastructure of liver & reducing the liver injury. Several reports support the taurine’s ability to protect liver function thereby restoring the blood levels of urea, the metabolite of urea cycle (Doğru-Abbasoğlu, 2001; Redmond et al., 1996).

4.5 Glucose metabolism

4.5.1 Liver Glycogen

Glycogen is a molecule that functions as the secondary long-term energy storage in animal cells. It is made primarily by the liver through glycogenesis. In the liver hepatocytes, glycogen can comprise up to 8% of the fresh weight and it plays an important role in the glucose cycle. Glycogen forms an energy reserve that can be quickly mobilized to meet a sudden need for glucose. In the present study, a significant (p<0.001) (Fig 4.5.1.1) decrease in the liver content of glycogen was noted in Group III rats intoxicated with D-GalN when compared to normal Group I rats. This is in agreement with similar reports. Hou et al., 2008 describe a decrease in hepatic glycogen stores of rats upon D-GalN administration in their study. Following D-GalN injection, the hepatic carbohydrate metabolism was greatly altered to maintain plasma glucose concentration. Glycogen dropped during the first hours, remaining low for up to 48 hr (De Oliviera et al., 1992).
Administration of group IV rats with taurine significantly (p<0.001) elevated the levels of glycogen (Fig 4.5.1.1) in liver when compared to Group III D-GalN-intoxicated rats. Several other reports support this observation (De Oliveira et al., 1992 and Mourelle & Meza 1989). The ability of taurine to restore the levels of glycogen in liver may in part be explained by its inhibitory effect on the activity of the glycogenolytic enzyme glycogen phosphorylase that catalyzes the breakdown of glycogen. In the present study D-GalN intoxicated Group III rats caused a significant (p<0.001) rise in the activity of the glycogen phosphorylase (Fig 4.5.2) resulting in depletion of the hepatic glycogen stores. This trend may be to compensate for the hypoglycaemia seen after D-GalN injection as reported earlier (Anandan et al., 2000 and Yamamoto et al., 1995).

Prior administration of taurine in rats of Group IV injected with D-GalN has resulted in significant (p<0.001) decrease in the hepatic activity of glycogen phosphorylase (Fig 4.5.1.2) when compared to Group III D-GalN intoxicated rats. This effect of taurine aids in enhancing the glycogen stores in the liver (Lapson et al., 1983). Taurine is reported to cause the stimulation of glycolysis and glycogenesis and the later was shown to be promoted because of the increase in glycogen synthase I and decrease in glycogen phosphorylase A activity. These effects of taurine were shown to be dependent on insulin concentration, suggesting a link between the two substances.

4.5.2 Glucose metabolism (glucose, glycolysis and gluconeogenesis)

Taurine is involved in many important biological functions including osmoregulation, inhibition of protein phosphorylation and calcium modulation. It is found at high concentrations within pancreatic islets (Bustamante et al., 1998). Taurine reduces the rate of apoptosis (Merezak et al., 2001) and acts on DNA synthesis, preventing abnormal development of the endocrine pancreas (Boujendar et al., 2003). Taurine has important
effects on insulin secretion. In guinea pigs with hyperglycemia, taurine administration significantly decreased blood glucose levels (Kaplan et al., 2004). Moreover, it has been shown that taurine increases glucose sensitivity cells by enhancing mitochondrial metabolism, (Han et al., 2004) at least partially by acting on the mechanism for Ca$^{2+}$ sequestration into the mitochondrial matrix (Lee et al., 2004). Taurine increases glycogen synthesis, glycolysis and glucose uptake in the liver and heart of adult rats (Lapson et al., 1983 and Kulakowski & Maturo 1984). Finally, taurine antioxidant properties protect pancreatic beta-cells against stress oxidative-induced decrease in function observed in some pathophysiological conditions (Oprescu et al., 2007). These actions indicate that taurine is involved in distinct central and peripheral processes necessary for the control of glucose homeostasis.

In the present study there was a marked (p<0.001) decrease in the levels of plasma glucose (Fig 4.5.2.1) in group III rats when compared to normal Group I rats. This is in agreement with similar published reports. In D-GalN-induced hepatic failure hypoglycemia is a marked feature. It is attributed to the depletion of glycogen stores in liver injury due to enhanced glycogenolysis. Also there is increase in the rate of glycolysis, the reason being hyperinsulinemic condition brought about by D-GalN injection. Ozeki et al., (1982) report that intraperitoneal injection of a single dose of galactosamine hydrochloride resulted in remarkable decreases of glycogen and UDPG in severely damaged liver which may be the cause of hypoglycemia

Administration of Group IV rats with taurine has resulted in rise in blood glucose levels (Fig 4.5.2.1) to normal levels when compared to Group III D-GalN-intoxicated rats. Though taurine is reported to decrease the concentrations of glucose, and increase the contents of insulin, C-peptide, and glycogen in the liver in many studies associated with experimental diabetes mellitus and these effects combine to make taurine hypoglycemic, taurine in this
study has elevated glucose levels. This may be due to taurine’s potential to counteract the D-GalN–induced aberrations and its overall positive effect on glucose homeostasis and its influence on all pathways of glucose metabolism that occur in the liver.

The activities of hexokinase and glucose-6-phosphate dehydrogenase were assayed to delineate the effect of taurine on glycolysis in D-GalN induced hepatic failure. The two enzymes catalyze rate limiting reactions in glycolysis and their activities are reported to be increased in D-GalN-induced fulminate hepatic failure (Anandan et al., 2000). The present study too describes a similar trend. There is a significant (p<0.001) rise in the levels of hexokinase (Fig 4.5.2.2) and glucose-6-phosphate dehydrogenase (Fig 4.5.2.3) in the Group III D-GalN-injected rats when compared to normal Group I control rats. Topaloglu et al., (1996) showed that galactosamine altered glucose transport and induced hypoglycemia and a high mortality in ten-day-old rats treated with a low dose of endotoxin. Galactosamine reduces tissue glucose uptake, depletes glycogen stores; factors which reduce the reserve potential of glycolysis. Administration with taurine in Group IV rats has resulted in a significant (p<0.001) decline in the activities of the enzymes (Fig 4.5.2.2 & fig 4.5.2.3) when compared to D-GalN-intoxicated Group III rats.

Glucose-6-phophatase and Fructose-1,6-bis phosphahatase are enzymes catalyzing rate limiting reactions in gluconeogenesis. Their activities were measured to describe the effect of taurine on D-GalN induced hepatic failure. Gluconeogenesis is reported to be taking place at a reduced rate in D-GalN intoxicated rats. Severe hepatic damage with massive necrosis causes a decrease in the activities of glucose-6-phosphatase and fructose-1, 6-diphosphatase as reported earlier (Ozeki et al., 1982). Therefore, glucose release from liver into the blood stream decreases and the inhibition of gluconeogenesis occurs. The present study also shows similar results. There is a significant (p<0.001) decrease in the activity of glucose-6-phosphatase (Fig 4.5.2.4) and fructose-1,6 bis phosphatase (Fig 4.5.2.5) in Group III rats.
intoxicated with D-GalN when compared to normal group I rats. It has been demonstrated that in FHF-induced rat livers there was reduced amino acid uptake, a switch from gluconeogenesis to glycolysis, causing an effective decrease in gluconeogenesis when compared with normal fasted rat livers. Mass-balance analysis showed that hepatic glucose synthesis was inhibited as a result of a reduction in amino acid entry into the tricarboxylic acid cycle by anaplerosis (Arai et al., 2001).

Administration with taurine in Group IV rats has resulted in a significant (p<0.001) (Fig 4.5.2.4) & (Fig 4.5.2.5) increase in the activities of the enzymes when compared to D-GalN-intoxicated Group III rats. In the present study taurine has restored glucose homeostasis by regulating glycolysis, and gluconeogenic rates and elevated glycogen levels in the liver by inhibiting glycogenolysis. The activities of the rate limiting enzymes (hexokinase & glucose-6-phosphate dehydrogenase) in glycolysis are lowered and that of gluconeogenesis (glycogen phosphorylase) is enhanced to regularize the blood glucose levels. During the last years, several studies have shown that taurine is involved in different central (strict regulation of insulin synthesis and secretion) and peripheral (peripheral metabolic effects of insulin) processes necessary for the control of glucose homeostasis ((Bustamante et al., 1998, Merezak et al., 2001, Boujendar et al., 2003, Kaplan et al., 2004, Kulakowski & Maturo 1984 etc), however, the key events underlying effects of taurine on blood glucose levels remain unknown (Nandhini et al., 2004). Taurine appears to act by regulation of the expression of genes required for glucose-stimulated insulin secretion depending on the glycemic state (Carneiro et al., 2009).

4.6 Protein & glycoprotein components

The levels of protein were significantly (p<0.001) lower in the plasma (Fig 4.6.1) and liver (Fig 4.6.2) of Group III rats intoxicated with D-GalN when compared to normal Group I rats.
The decrease observed in the present study is in accordance with studies reported earlier. Koj & Dubin, 1978 showed that galactosamine administered 30 min before [3H] lysine significantly inhibited the incorporation of the label into liver proteins, and plasma proteins. The administration of D-GalN leads to inhibition of protein and glycoprotein secretion by rat liver. Previous researchers have established that the disturbed secretion of proteins and glycoproteins was due to cumulative effects of galactosamine. Protein synthesis is inhibited in the following manner: 1. D-GalN traps uridine required for RNA formation and subsequent protein synthesis by forming UDP-galactosamine. 2. Galactosamine metabolites inhibit galactosyltransferase activity the enzyme that catalyzes the formation of UDP-galactose that is essential for RNA synthesis. 3. UDP-galactosamine progressively replaces UDP-galactose.

Administration of Group IV rats with taurine has restored (p<0.001) the levels of protein in plasma and liver ((Fig 4.6.1 & Fig 4.6.2) when compared to D-GalN-intoxicated rats Group III rats. Glycosylation of proteins is initiated when the polypeptide chain is still attached to the ribosomes (Gentzsch & Tanner 1997) and is completed in Golgi apparatus (Keenan, 1998). During the induction of galactosamine hepatitis (Sugiama et al., 1999), morphological alterations of the endoplasmic reticulum were observed and alterations of glycoprotein synthesis were found. In the present study the carbohydrate residues hexose, hexosamine and sialic acid were assayed as an index of the glycoprotein content. There was a significant (p<0.001) decline in the contents of hexose (Fig 4.6.3 & Fig 4.6.4), hexosamine (Fig 4.6.5) and sialic acid (Fig 4.6.6 & Fig 4.6.7) in the liver and plasma of Group III rats intoxicated with D-GalN when compared with normal Group I rats. These results agree with studies reported previously. Bolmer & Kleinerman (1987) in a study of effect of D-GalN on glycoprotein alpha-1 antitrypsin report that the hepatotoxin had drastically lowered the plasma protein-bound carbohydrate content: sialic acid decreased by 60%, neutral sugars decreased by 43% and amino sugars decreased 38%. Monnet et al., (1985) demonstrated that
D-GalN injection caused a decline in the content of sialic acid residue of alpha 1 acid glycoprotein. Ozeki et al., (1982) describe that D-GalN through inhibition of key hepatic enzymes of glycoprotein synthesis reduce the content of glycoprotein in liver.

Administration of Group IV rats with taurine has restored (p<0.001) the levels of protein-bound carbohydrate residues in plasma and liver (Fig 4.6.3, Fig 4.6.4, Fig 4.6.5, Fig 4.6.6 & Fig 4.6.7) when compared to D-GalN-intoxicated rats Group III rats. Taurine, a potent organic osmolyte regulates cell hydration and in conditions of stress enhances hydration that stimulates protein synthesis. Hagar (2004) reports that taurine increased protein synthesis in liver as part of amelioration of oxidative stress-induced hepatotoxicity in rats. Taurine, through its role in osmoregulation, membrane stabilization, oxidative damage prevention, calcium modulation etc provides a comprehensive protection to the hepatocytes and prevents the D-GalN-mediated aberration in liver function. As part of this hepatoprotection, protein synthesis improves in the liver that explains the restoration of protein and glycoprotein levels in liver and plasma.

4.7 Lipid metabolism

Fulminant hepatic failure has several manifestations and a derangement in lipid metabolism is one of the important ones. Fat accumulation takes place in the hepatocytes as seen in liver morphology of D-GalN-induced liver toxicity. This develops either due to excessive supply of lipids to the liver or interference with lipid clearance. In galactosamine-induced hepatic failure, the liver cells become fibrotic leading to excess accumulation of fat. The pathogenesis is multifactorial, reflecting complex biosynthetic, enzymatic and catabolic derangement in lipoprotein metabolism.

Significant raises occurred in the levels of blood and liver lipids of D-GalN-intoxicated rats of Group III when compared to normal Group I rats. The increase noticed in the levels of
circulatory lipids is a well-known evidence for the hyperlipidemic nature of D-GalN (Ravikumar et al., 2005). The accumulation of lipids in the liver of rats treated with D-GalN could be one of the factors responsible for the liver functional disorders. Lipid accumulation in the liver tissue may provide increased substrate for peroxidative damage, which is one of the major causative factors involved in the D-GalN-induced injury to the hepatocyte membrane (Nagoshi et al., 1994; Matyushin, 1983).

4.7.1 Cholesterol

In the present study a significant (p>0.001) increase was observed in the levels of serum total cholesterol (Fig 4.7.1.1), VLDL-cholesterol (Fig 4.7.1.2) and LDL-cholesterol (Fig 4.7.1.3) in the Group III rats when compared to Group I normal rats. Also HDL was markedly (p>0.001) (Fig 4.7.1.4) reduced in Group III rats indicating the hypercholesterolemic action of D-GalN. There was also a notable (p>0.001) increase (Fig 4.7.1.5) in the levels of liver cholesterol in Group III. This is in agreement with other studies (Sathivel et al., 2008) where it was reported that D-GalN-toxicity causes accumulation of fat in liver and increases lipid levels in serum.

Administration with taurine has caused the blood levels of total cholesterol and LDL cholesterol to significantly decrease and HDL cholesterol to increase in Group IV rats when compared to D-GalN-intoxicated Group III rats. Several previous studies support these results (Yanagita et al., 2008 and Lombardini & Julius, 2006). In the liver, taurine plays a role in conjugation of bile acids. The hypocholesteolemic effect of taurine in rats (Sugiama et al., 1989 & 1984), mice (Kamata et al., 1996, Yamanaka et al., 1986), and humans (Zhang et al., 2004) has been established, but the mechanisms by which taurine decreases plasma cholesterol is not well defined. Taurine increases bile acid synthesis (Yamanaka et al., 1986) concomitant with increase in activity and mRNA expression of cholesterol 7α–hydroxylase (Ebihara et al., 2006). It is implied that increased conversion of cholesterol into bile acids
through stimulation of cholesterol $7\alpha$-hydroxylase, a rate-limiting enzyme in hepatic bile acid synthesis, may be the primary mechanisms responsible for the hypocholesterolemic action of taurine. The increase in cholesterol elimination from the liver results in the reduction of hepatic cholesterol pools, an event which may lead to up-regulation of cholesterol synthesis and LDL receptor activity to compensate for cholesterol depletion (Murakami et al., 2002). In cholesterol homeostasis, cholesterol clearance from the bloodstream through hepatic LDL receptor is responsible for reduced plasma LDL cholesterol levels. In effect taurine lowers liver tissue cholesterol and circulating levels of total and LDL cholesterol.

### 4.7.2 Triglycerides, Free fatty acids and triglyceride lipase

Hypertriglyceridemia has been reported to be associated with conditions where severe liver injury occurs due to inability of the liver to metabolize fat optimally (Borowsky et al., 1980). In the present study, a significant (p<0.001) increase was observed in the levels of triglycerides and free fatty acids in the plasma and liver tissue of Group III D-GalN-administered rats as compared to that of Group I control rats (Fig 4.7.2.1, 4.7.2.2, 4.7.2.3 & 4.7.2.4). This concurs with earlier reported studies, which showed that injection of D-GalN lead to fatty accumulation in the liver tissue (Kajikawa et al., 2009). The administration of D-GalN has been reported to cause liver parenchymal damage and necrosis as a result of which the metabolism of fat is grossly impaired (Gujral et al., 2003). The normal physiological process of turnover of triglycerides from peripheral tissues like adipose tissue delivers free fatty acids to the liver (Steinberg 1976). But the injured liver fails to metabolize and assimilate them which apparently is the cause of rise in serum and liver tissue FFA and triglycerides. Liver is also a site for synthesis and assimilation of lipoproteins like LDL and VLDL through which triglycerides are secreted into circulation (Shen et al., 1998), failure of
which causes their rise in the liver tissue. A significant (p<0.01) increase in the activity of hepatic triglyceride lipase was also observed in D-GalN-treated rats as compared to that of Group I controls (Fig 4.7.2.5), indicating increased lipolysis in the liver tissue.

The liver derives a significant portion of its fatty acid substrates as free fatty acids derived by lipolysis from adipose tissue. Although lipid availability is important for the liver, excess levels of fatty acids in hepatocytes can be deleterious (Reddy & Rao 2006). Though the liver can utilize free fatty acids for its energy requirements, the excess free fatty acid may be used for the synthesis of triglycerides, resulting in hypertriglyceridemia (Iritani et al., 1976). In the present study, prior treatment with taurine significantly (p<0.001) prevented the D-GalN-induced elevation in the levels of triglycerides and free fatty acids in plasma and liver tissue of Group IV rats as compared to that of Group III rats. Taurine also lowered the activity of hepatic triglyceride lipase that may have led to decreased lipolysis in liver. Taurine treatment has successfully lowered the lipid components in the serum and liver tissue but compared to its effect on LDL cholesterol and total cholesterol, its ability to reduce triglyceride concentration is modest. The hepatoprotective effect of taurine is probably related to its ability to inhibit the accumulation of lipids in the liver by its antilipidemic property (Militante & Lombardini, 1996). One of the advantages of taurine is that, unlike synthetic antilipidemic agents it is a naturally occurring compound that is both produced endogenously and naturally occurring in foods. Studies (Mozaffari et al., 2006; Chen et al., 2004), indicate that pathology develops when the animal is depleted of its taurine stores either by taurine deficient diet or through taurine antagonists.

4.7.3 Phospholipids

Phospholipids concentration in liver tissue (Fig 4.7.3.1) of D-GalN-administered animals was significantly (p<0.001) lower than that of control animals. However in plasma their
levels show a significant (p<0.001) rise (Fig 4.7.3.2) in D-GalN-administered animals than that of control animals. It has been reported that D-GalN injury related alterations in lipid composition of hepatic tissue appears to occur due to the destruction of hepatocyte membrane lipid bilayer (Vinogradova et al., 1998; Dvoi et al., 2003). The intracellular calcium (Ca^{2+}), an inducer of phospholipase A_2, which degrades membrane phospholipids, has been reported to rise in D-GalN-induced hepatic injury (Komano et al., 2009). Phospholipase A_2 acts on phospholipids, with the release of free fatty acids. Hence, the significant elevation noticed in the levels of free fatty acids in plasma and liver tissue of D-GalN-treated rats might be due to enhanced breakdown of membrane phospholipids liver by the lipolytic action of phospholipase A_2 (Petkova et al., 1987), which could be very likely the biochemical basis for the cell injury. Studies by Steigen et al., (1992) showed that exogenous lipases attacked energy-depleted cells in culture and had no effect on normal cells. Previous studies (Lin et al., 2009; Matyushin et al., 1983) suggest that lipid accumulation and peroxidation in the liver may be key events that determine D-GalN-induced hepatic failure. Further support for this conclusion comes from reports showing that D-GalN depletes antioxidant protection of the liver (Shi et al., 2008), and therefore may provide conditions conducive to lipid peroxidation.

It is possible that lipid peroxides by their destructive action on the mitochondrial membranes cause instability and contribute to intracellular calcium accumulation (Farber et al., 1977) and phospholipase activation. This presumption is further supported by studies in cultured cells in which inhibition of fatty acid accumulation by phospholipase inhibitors protected the cells from calcium overload and morphological damage (Wu et al., 1999). The results of the current investigations showed that the prior administration of taurine significantly (p<0.001) prevented the D-GalN-mediated degradation of membrane phospholipids, establishing its membrane stabilizing effect. One particular study (Li et al.,
2009) presents data signifying the important role of taurine in stabilizing the mitochondrial membrane and its environment preventing escape of the reactive compounds formed in the mitochondrial environment and calcium ions and thus indirectly protecting the phospholipids bilayer.

4.7.4 Fatty acid composition

Fatty acids play an integral role in determining the structural and functional properties of cellular and subcellular membranes of the liver. Their influence on fluidity and stability of membrane structure markedly impacts on membrane functions such as transport of the ions and substrates, and maintenance of membrane potential etc, which are intrinsic to liver function (Wahle, 1983). In addition to the structural and functional roles played within the cell membrane, fatty acids and associated lipids are also recognized as regulatory molecules with roles in cell signaling, as second messengers in transduction, and as effectors in apoptosis (programmed cell death) in response to oxidative stress (Epand et al., 2004; Tang et al., 2002). Apoptosis plays a prominent role in the hepatocyte loss that occurs in fulminant hepatic failure (Ryo et al., 2000; Yan et al., 2009).

In the present study, significant decreases were observed in the level of saturated fatty acids (C14:0, C16:0 and C18:0) in D-GalN-administered rats as compared to normal rats (Fig 4.7.4.1). This is in accordance with earlier reported study (Bollard et al., 2002), which indicates that the oxidation of saturated fatty acid is associated with diminished hepatocyte function. Saturated long chain fatty acid substrates such as palmitate (but not mono-unsaturated fatty acids) induce apoptosis in rat neonatal hepatocytes (Listenberger et al., 2001). Interestingly, in contrast to saturated fatty acids, slight elevation was noticed in the levels of C16:1, C18:1, C18:2, C20: 4 in Group III D-GalN-induced rats as compared to that of Group I control rats. The increase observed in the levels of unsaturated fatty acids might
be either due to the residual uptake of fatty acids from extracellular sources such as blood and adipose tissue and their accumulation caused by reduced use in the mitochondrial oxidation (Mangeney et al., 1985) or due to the action of phospholipases on membrane lipids (Petkova et al., 1987). During liver injury, fatty acid homeostasis is severely disturbed. Accumulation of nonesterified fatty acids and their metabolites occur because of diminished mitochondrial oxidation and respiratory chain activity reported in D-GalN toxicity (Devaki et al., 2009). Moreover the accumulation of nonesterified arachidonic acid signals the beginning of a chain of events that include eicosanoid synthesis (Liu et al., 1992). Reports by Liu & Chang (2009) show that prostaglandins and leukotrienes derived from arachidonic acid induce inflammatory signals in liver tissue. Ion channels (sodium and calcium) channels become hyperactive and fatty acids act as messenger molecules that regulate these ion channels (Petrou et al., 1995).

Polyunsaturated fatty acids are major components of membrane phospholipids and play a key role in membrane functions (Portero-Otín et al., 2001). In the present study, a significant reduction was observed in the levels of polyunsaturated fatty acids (EPA and DHA) (Fig 4.7.4.2) in the liver tissue of D-GalN-treated Group III rats as compared to Group I control rats. Epidemiological studies indicate that EPA and DHA have been reported to possess cytoprotective action (Sangiovanni & Chew 2005; Sellmayer & Koletzko, 1999). The protective effects of n-3 PUFA are attributable to their direct effects on vascular endothelial and smooth muscle cell functions (Abeywardena and Head, 2001). The greater effect of DHA was considered to be due to its greater ability to decrease membrane cholesterol content and the cholesterol/phospholipids molar ratio and also to its greater ability to elevate the unsaturation index in the plasma membrane (Dusserre et al., 1995). These physicochemical alterations in the membrane properties may directly or indirectly influence functions of membrane-bound proteins such as receptors, GTP binding proteins, ion channels and various
enzymes. Persistent cellular oxidative stress and enhanced peroxidation of PUFAs, leading to macromolecular and membrane damage and disruption of signaling pathways are known to stimulate hepatocyte injury (Bartsch & Nair, 2004 and Behn et al., 2007). Thus the decline in the level of these fatty acids might have rendered the liver more susceptible to D-GalN-induced injury.

The ratio of n6 to n3 fatty acids were significantly higher (p< 0.05) in Group III D-GalN-induced rats (Fig 4.7.4.3) when compared to Group II taurine supplemented rats. A lower ratio is favorable as had been observed in the diet of the early man. Modern day diet has a severely skewed n6 to n3 ratio that has significant negative ramifications on man’s health. Taurine supplementation in Group IV has slightly reduced the ratio which is a positive effect.

Prior treatment with taurine significantly prevented the abnormalities in fatty acid composition in bilayers Group IV rats when compared to Group III rats. Taurine may have preserved the levels of saturated fatty acids and polyunsaturated fatty acids by protecting the lipid of cellular and subcellular membranes from peroxidative damage. Taurine might have ameliorated the D-GalN-induced liver injury either by strengthening hepatocyte membrane by its membrane stabilizing action and/or by relieving the oxidative stress, thereby protecting the lipid bilayer from peroxidation.

4.8 Tissue defense and antioxidant status

Oxidative stress occurs in most if not all human diseases (Favier 2006). Oxygen, indispensable for maintaining life, sometimes becomes toxic and results in the generation of most aggressive agents, reactive oxygen species (ROS) or free radicals. Free radicals are generated by biochemical redox reactions that occur as a part of normal cell metabolism and in the course of free radical-mediated diseases. The high reactivity of ROS may trigger a host
of disorders in body resulting in tissue damage and necrosis in many instances (Bergamini et al., 2004). Biological membranes are sensitive to lipid peroxidation induced by reactive oxygen species. The oxidation of unsaturated fatty acids in biological membranes causes impairment of membrane function, decrease in membrane fluidity, inactivation of membrane receptors and enzymes, increase of non-specific permeability to ions, disruption of membrane structure and inhibition of metabolic processes. (Nigam & Schewe, 2000). Oxidative stress is one of the causes of hepatocyte damage induced by D-GalN, as increased production of reactive oxygen species has been reported in vivo (Yoshikawa et al., 1982) and in vitro (Quintero et al., 2002).

The body on account of susceptibility to oxidative insult is provided with an efficient antioxidant system which decreases concentrations of the harmful oxidants in the tissues. A series of enzymes act as scavenging systems which include superoxide dismutase (SOD), catalase, glutathione-S transferase, and glutathione peroxidase and glutathione reductase. These enzymes are the first line of defense against reactive oxygen species and are generally referred to as primary antioxidants (Michiels et al., 1994). SOD, CAT and GPX constitute a mutually supportive team of defence against reactive oxygen species. SOD is a metalloprotein and is the first enzyme involved in the antioxidant defence by lowering the steady-state level of O$_2^-$ converting it into H$_2$O$_2$ and water. CAT is a hemeprotein, localized in the peroxisomes that catalyses the decomposition of H$_2$O$_2$ to water and oxygen and thus protecting the cell from oxidative damage by H$_2$O$_2$ and OH$^-$. GPX is a seleno-enzyme that catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide.

The 2$^{nd}$ line of defence against free radical damage is the presence of antioxidants such as glutathione (Furst, 2009) a cellular tripeptide, (L-glutamyl cysteinyl glycine) and a major non-protein thiol. Glutathione is a part of an antioxidant defense system that plays a crucial
role in coordinating the body’s cellular protection against reactive free radicals (Anandan et al., 1999b; Meister & Anderson, 1983). Perturbation of GSH status of a biological system has been reported to lead to serious consequences (MacDonald, et al., 1984). Oxidative stress occurs as a consequence of imbalance between the formation of free radicals and inactivation of these species which through a series of events deregulates the cellular functions leading to various pathological conditions.

4.8.1 Lipid peroxidation

ROS are toxic to cells because they can react with biological membranes and most cellular macromolecules, including proteins, lipids, and DNA (Loguercio & Federico 2003). They peroxidize unsaturated lipid molecules that occur in hydrophobic core of bio-membranes, producing lipid peroxidation products, TBARS at the site of oxidative stress. Lipid peroxidation is central to development of liver injury induced by drugs and it is one of the several mechanisms by which galactosamine is reported to cause fulminate hepatic failure (Mizuoka et al., 1999). There is a significant (p<0.001) (Fig 4.8.1.1) increase in the ROS formation measured in terms of TBARS in the liver of Group III galactosamine-induced hepatitis rats in comparison with that of Group I control rats. This is in conformity with several studies which also report that lipid peroxidation is one of the key factors for galactosamine-stimulated hepatic failure (Wang et al., 2008; Lim et al., 2000; Thabrew et al., 1995; Abdul-Hussain & Mehendale, 1992). Lipid peroxidation is strictly prevented and regulated by multiple defense mechanisms involving ROS scavenging enzymes and small antioxidant molecules in normal tissues. In the liver tissue of galactosamine-intoxicated rats the rise in lipid peroxidation may be due to the collapse of this defense system.

Even as the mechanism of galactosamine hepatotoxicity is unclear, one possible course is the inhibition of protein synthesis by D-GalN in hepatocytes. D-GalN causes a decrease in
the level of uracil nucleotides followed by the inhibition of RNA synthesis and disturbance of
the biosynthesis of glycoproteins leading to the impaired formation of cellular membranes
(Decker & Keppler 1974). These changes in the structure of cellular membranes may
stimulate lipid peroxidation generating lipid radicals and eventually to short-chain aldehydes
and hydroxy alkenals that eventually culminate in the formation of conjugated dienes,
malondialdehyde, and alkanes. Some investigators (Sun et al., 2003) have reported that lipid
peroxidation may play a central role in the liver damage due to galactosamine and in several
studies it was shown that MDA and TBARS levels as indicators of lipid peroxidation were
found increased significantly in liver homogenates of D-GalN treated rats. Sun et al., 2003
report that the concentration of lipid hydroperoxides in liver increased significantly 24 hr
after D-GalN administration and in contrast, the concentration of vitamin C and reduced
 glutathione in the liver decreased significantly 18 and 24 hr after D-galactosamine
administration. These results suggest that D-GalN induces severe oxidative stress in the liver,
leading to extensive necrosis.

It is evident from the present study that taurine administration to rats in Group IV has
rendered protection against D-GalN-induced oxidative stress; there is significant (p<0.001)
(Fig 4.8.1.1) reduction in the levels of TBARS in plasma and liver tissue which is a clear
indication of antioxidant properties of taurine. Numerous studies support the potential of
taurine as an antioxidant molecule (Balkan et al., 2001, Timbrell et al., 1995). The effects of
acute oxidative stress on the ultra structure of sinusoidal endothelium, space of Disse,
hepatocytes and Kupffer cells in perfused rat liver have been studied previously by Cogger et
al., 2004. They successfully demonstrated the alterations brought about by taurine in injured
hepatocytes. Tasci et al., 2008 have shown beneficial effects of taurine on histopathology and
oxidative stress parameters in a rat model of CCl₄-induced liver fibrosis where remarkable
histopathological improvement in taurine treated animals subjected to hepatotoxin was
observed, and this was associated with oxidative stress reduction. Taurine is also reported to have beneficial effects in various physiological and pathological conditions (Hanna et al., 2004, Roysomuti et al., 2003, Della Corte et al., 2002, Timbrell et al., 1995) by mainly diminishing production of reactive oxygen species (ROS). Hepatoprotective feature of taurine is attributed to its inhibitory activity on generation of ROS, which are known to play an important role in hepatic injury. The other major function of taurine is to trap chlorinated oxidants by producing the nontoxic, long-lived taurine-chloroamine, and thus protect the cell from destruction during processes that produce oxidants (Ogino et al., 2009). Because taurine is exceptionally abundant in the cytosol of inflammatory cells, especially in neutrophils, taurine will travel with the migrating neutrophils to the damaged organs to combat free radicals (Marcinkiewicz et al., 1995). Taurine haloamines taurine chloramines and taurine bromamine have significant role in mitigation of inflammation and oxidative stress (Marcinkiewicz et al., 2006).

4.8.2 Total reduced glutathione

GSH is a major non-protein thiol in living organisms, which plays a central role in coordinating the body’s antioxidant defense processes and detoxification. Glutathione is a component of a pathway that uses NADPH to provide cells with their reducing milieu. This is essential for (a) maintenance of the thiols of proteins and of antioxidants (e.g. ascorbate, alpha-tocopherol), (b) reduction of ribonucleotides to form the deoxyribonucleotide precursors of DNA, and (c) protection against oxidative damage, free radical damage, and other types of toxicity. Perturbation of GSH status of a biological system reflects defunct oxidant defense system and has been reported to lead to serious consequences (Kim et al., 2006). In the present study, a significant (p<0.001) decrease (Fig 4.8.2.1) was observed in the levels of hepatic GSH content in galactosamine administered Group III rats when
compared to Group I normal control rats. Similar observations were reported by other investigators (Ohta et al., 2007), which establishes the fact that GSH depletion occurs in the liver of rats after D-GalN administration. A study reported that D-GalN suppresses resynthesis of GSH in rat primary hepatocyte culture through a direct inhibitory effect on GSH synthetase. (Mc Millan & Jollow, 2006). Mc Millan & Jollow reported that in D-GalN toxicity there is a slow but extensive depletion in hepatic reduced glutathione (GSH) (McMillan and Jollow, 1992) which may also contribute to galactosamine toxicity because administration of low molecular weight sulfhydryl compounds (which restore GSH content by stimulating GSH synthesis) diminished the toxic response.

Glutathione is a potent cellular reductant with a broad redox potential. It directly quenches reactive hydroxyl free radicals, other oxygen-centered free radicals, and radical centers on DNA and other biomolecules (Lomaestro & Malone 1995) and serves as a storage and transport form of reduced sulphur (Kidd, 1997). GSH is a primary protectant of skin, lens, cornea, and retina against radiation damage, and the biochemical foundation of P450 detoxication in the liver, kidneys, lungs, intestinal epithelia, and other organs.

GSH is the essential cofactor for many enzymes which require thiol-reducing equivalents, and helps keep redox-sensitive active sites on enzymes in the necessary reduced state (Weber 1999). Higher-order thiol cell systems the metallothioneins, thioredoxins, and other redox regulator proteins are ultimately regulated by GSH levels and the GSH/GSSG redox ratio. GSH/GSSG balance is crucial to homeostasis, stabilizing the cellular biomolecular spectrum, and facilitating cellular performance and survival (Lomaestro & Malone 1995). GSH and its metabolites also interface with energetics and neurotransmitter syntheses, through several prominent metabolic pathways (Gul et al., 2000).

In view of the numerous physiological roles that GSH plays in a living cell, a decline in GSH levels would deprive the cells of many of its specific roles exacerbating the oxidative
damage. In a study researching the effect of green tea on D-GalN-induced liver injury it was demonstrated that D-GalN induced hepatic hypoxia and triggered ROS production from affected hepatocytes, infiltrated leukocytes, and activated Kupffer cells all of which led to hepatocyte apoptosis (Wada et al., 1999). In cells under attack from ROS, there is enhanced consumption of antioxidant molecules like GSH and antioxidant enzymes (Comporti, 1985). The reduction in GSH may be also due to enhanced degradation, reduced synthesis and reduced rate of reformation from GSSG the oxidized state. NAD(P)H acts as hydrogen ion donor in the re-reduction of GSSG to GSH, catalyzed by glutathione reductase. Thus NADPH, by the process of maintaining antioxidative power of glutathione, acts as an antioxidant (Kirsch & de Groot, 2001). Levels of NADPH are reported to be drastically lowered in the state of oxidative stress induced by D-GalN (Hu et al., 1992).

In the present study the hepatic and plasma content of GSH was significantly (p<0.001) increased in taurine treated Group IV rats when compared to D-GalN-injected rats (Fig 4.8.2.1). Antioxidants have the ability to scavenge the free radicals and inhibit the peroxidation of phospholipids thus protecting the membrane from damage. Antioxidants like, vitamins C and E, GSH and GSH augmenting agents exert cytoprotective effect in experimental conditions that produce oxidative stress. Similarly taurine is an effective naturally occurring antioxidant in the cell that is expected to show a similar effect. In a study reported by Hagar, 2004 taurine showed potent antioxidant effect against cyclosporine A-induced oxidative stress by elevating hepatic GSH content. GSH depletion exacerbates D-GalN hepatotoxicity rendering the hepatocyte more susceptible to oxidative stress (Mc Millan & Jollow, 2006). The prior administration of taurine to rats intoxicated with D-GalN replenished GSH content in the plasma and liver tissue of D-GalN-intoxicated rats which reveals the antioxidant capacity of taurine. Explanations of the possible mechanisms by which taurine protects the integrity of the hepatic tissue prevention of GSH depletion,
destruction of free radicals and counteracting the reactive oxygen species mediated lipid peroxidation (Tabassum et al., 2006). Taurine strengthens the endogenous antioxidant defenses to fight ROS damage and restore the healthy state of the cell by neutralizing the reactive species.

4.8.3 GSH dependent antioxidative enzymes

The levels of GSH dependent antioxidative enzymes GPX and GST are significantly (p<0.001) lowered in the liver of Group III rats intoxicated with D-GalN when compared to normal Group IV rats (Fig 4.8.3.1 & Fig 4.8.3.2). This is in agreement with other reports (Vimal & Devaki 2004; Sreepriya et al., 2001). GPX is a selenoprotein that has an essential role in protecting tissues against the pro-oxidant-induced lethality and hepatic necrosis (Halliwell & Gutteridge, 1999; Cheng et al., 1999). The biochemical role of the enzyme is to catalyze the reaction between GSH and H₂O₂ to form water and oxidized glutathione. The reduced form of glutathione is regenerated by the action of the enzyme glutathione reductase in the presence of NADPH. GSTs, a family of cytosolic multifunctional proteins, are detoxifying enzymes present in all aerobic organisms that can act on both xenobiotics as well as endogenous reactive compounds of cellular metabolism (Kim et al., 2004). They catalyze the conjugation of glutathione with a variety of reactive electrophilic compounds, thereby neutralizing their active electrophilic sites and subsequently making the parent compound more water soluble for excretion. This explains their efficacy in combating D-GalN-induced oxidative stress. In addition to catalytic functions, the GSTs can also bind covalently/non-covalently to a wide number of hydrophobic compounds, such as haem, drugs and carcinogens to detoxify them. D-GalN administration causes hypoxia induced ROS generation and GSH depletion in the affected cells which concurrently causes the levels of GSH dependent enzymes GPX and GST to fall too (Neihorster et al., 1992). Oxidative stress...
induced by a wide type of drugs is reported to downregulate the GST family of supergenes (Romero et al., 2006), and a similar effect may be produced by D-GalN leading to lowered expression of GST group of enzymes.

Taurine has conferred considerable protection against oxidative stress induced by D-GalN by significantly (p<0.001) elevating the levels of GPX and GST in the present study. These results suggest that taurine has hepatoprotective effects against D-GalN-induced oxidative stress by inhibiting lipid peroxidation and maintaining an adequate level of GSH. Also taurine administration aided in maintaining optimal activity of GSH dependent enzymes, preserved the reducing milieu actions, all of these actions being the mechanisms by which taurine exhibits its underlying hepatoprotective effect. Among taurine’s emerging roles, is its effect on gene transcription (Gurujeyalakshmi et al., 1996) - to combat ROS attack, taurine may have a role in up regulating the genes for expression of GST and GPX.

**4.8.4 Antiperoxidative Enzymes**

Significant (p<0.001) decrease was observed in activities of the antiperoxidative enzymes CAT and SOD (Fig 4.8.4.1 & Fig 4.8.4.2) in the liver of D-GalN treated group III rats when compared to normal Group I rats. These results are in concurrence with other previous studies (Wu et al., 2009; Gezginci-Oktayoglu et al., 2008; Anandan & Devaki, 1999). SOD and CAT along with GPX form the first line of defence against ROS and are referred to as primary antioxidants. SOD essential to catalyze the dismutation of superoxide, protects cells from oxygen free radicals (Kojda & Harrison 1999; Anand et al., 1998). Three isozymes of SOD have been identified at the molecular level in mammals: intracellular Cu, Zn-SOD, mitochondrial Mn-SOD, and extracellular (EC)-SOD. Catalase, which decomposes H$_2$O$_2$ to water and O$_2$, is a widely distributed enzyme and is an important member of the cellular defense system against oxidative stress. Even if it is not strictly essential, the lack or malfunction of catalases may lead to severe defects, such as an increased susceptibility to
thermal injury (Leff, 1993), high rates of mutations (Halliwell & Aruoma, 1991) and, in higher organisms, inflammation (Halliwell & Gutteridge, 1990). Diagrammatic representation of the antiperoxidative enzymes is shown below. Reduction in the activities of these enzymes lead to the accumulation of $O_2^-$ and $H_2O_2$, which in turn can form hydroxyl radical (OH*) and bring about a number of reactions harmful to the cellular and subcellular membranes (Kalra et al., 1988). Free radical damage per se of the active sites of these two important antiperoxidative enzymes might be a possible cause of the decline in their activity in D-GalN intoxicated rats. The enzymes have amino acids arginine and histidine in their active sites that have an unpaired electron each and are susceptible to free radical damage (Datta et al., 2000).

Group IV rats that were on taurine supplemented diet showed a significant rise in the levels of antiperoxidative enzymes that signifies the protective effect of taurine in hepatic oxidative stress. Similar findings were reported by other researchers (Das et al., 2010). Thus, administration of taurine (100mg/kg body weight) protected the hepatic tissue from D-GalN-induced, acute oxidative stress. Taurine supplementation could have reduced lipid peroxidation by decreasing ROS which in turn could result in rise of the antiperoxidative enzymes (Nandhini et al., 2005). Attenuation in tissue lipid peroxidation may be a result of direct antioxidant action that scavenges or quenches oxygen free radicals intracellularly to block ROS cell death (Wu et al., 1999). Other investigators, have shown the beneficial effects of the ROS-scavenging capacity of taurine, specifically in relation to attenuation of lipid peroxidation, reduction of membrane permeability, and inhibition of intracellular oxidation in different cells (Chen, 1993 and Milei et al., 1992). Apart from taurine’s role as a scavenger of ROS, complex formation between sulphonic acid group (SO$_3^-$) to free metal ion species such as Fe$^{2+}$, Cu$^{2+}$ or oxidant metalloproteins has been reported (Tractman et al., 1992).
The beneficial effects of taurine as an antioxidant in biological systems have been attributed to its ability to stabilize biomembranes (Wright et al., 1986), scavenge reactive oxygen species (Wright et al., 1985) and reduce the production of malondialdehyde (MDA), an end-product of lipid peroxidation, from the unsaturated membrane lipids and a marker of oxidative stress (Huxtable, 1992). A lot of studies about the antioxidant effect of taurine in the lung (Banks et al., 1992), heart (Milei et al., 1992) and liver tissues (Nakashima et al., 1982) have been performed. Several mechanisms may play a role in taurine-mediated reduction in oxidative stress. Taurine was reported to protect cells by scavenging oxygen free radicals, by upregulating the antioxidant defenses, forming chloramines with HOCl, or binding free metal ions such as Fe^{2+} by its sulfonic acid group (Schaffer et al., 2003, Redmond et al., 1996). Because cysteine is a precursor of taurine and GSH, taurine supplementation may cause enhancement in GSH levels by directing cysteine into the GSH synthesis pathway (Yalçınkaya et al., 2009). Therefore, increased GSH levels after taurine treatment may play an additional role in decreasing oxidative stress.

4.9 Membrane bound ATPases

\( \text{Na}^+ , \text{K}^+ -\text{ATPase} \) is a plasma membrane-bound enzyme that provides the necessary electrochemical gradients of \( \text{Na}^+ \) and \( \text{K}^+ \) to maintain the cell volume and thus it plays a crucial role in homeostasis. It functions by exporting intracellular \( \text{Na}^+ \) and importing extracellular \( \text{K}^+ \) across the plasma membrane to provide energy for membrane transport of various metabolites taking part in special cell functions.

In the present study a significant (p<0.001) reduction was noticed in activities of the membrane-bound ATPases (Total ATPase, \( \text{Na}^+ \), \( \text{K}^+ \)-ATPase and \( \text{Ca}^{2+} \)-ATPase) in the liver of Group III D-GalN-induced fluminat hepatic failure rats when compared to Group I normal rats (Fig 4.9.1, 4.9.2 & 4.9.3). This observation is in conformity with a previous study,
where a severe derangement of subcellular metabolism and structural alterations in hepatocyte membrane upon D-GalN treatment were reported (Vinogradova et al., 1998). D-GalN intoxication leads to defective protein-lipid membrane bilayer formations due to defective glycoprotein synthesis (Petkova et al., 1987, Ozeki et al., 1982, El-Mofty et al., 1975). This triggers oxidative stress induction, disruption of membrane fluidity and decrease in Na\(^+\), K\(^+\)-ATPase and Ca\(^{2+}\)-ATPase activities by oxidation of thiol groups (Dobrota et al., 1999). Disturbance of the structure and permeability of cell membranes, including membranes of the endoplasmic reticulum and mitochondria leads to necrobiosis and cytolytic damage of the cells (Matyushin et al., 1983). Also in D-GalN toxicity, due to hypoglycemia, depressed glycolysis and tricarboxylic acid cycle, there is an acute shortage of energy in the form of ATP (Feng et al., 2007). Activity of Na\(^+\), K\(^+\)-ATPase declines rapidly in the absence of ATP (Wang et al., 2003). As a consequence an increase in extracellular K\(^+\) occurs and an influx of Na\(^+\), Cl\(^-\), and Ca\(^{2+}\) into the takes place. The initial increase in extracellular K\(^+\) concentration may spread rapidly, triggering depolarizations and reversal of the membrane transporters, leading to loss of cellular functions (Krick et al., 2001).

The ionic milieu of normal cells is maintained by the highly regulated Na\(^+\)/K\(^+\) ATPase or pump and the Ca\(^{2+}\) pump (Skou & Esmann, 1992). The Na\(^+\)/K\(^+\) ATPase catalyses the hydrolysis of ATP and couples it to the transport of Na\(^+\) and K\(^+\) across the cell membrane thereby generating the transmembranous Na\(^+\)/K\(^+\) gradient and resting membrane potential and maintaining cell volume, factors that play a crucial role in cell homeostasis (Baltz et al., 1997). It functions by exporting intracellular Na\(^+\) and importing extracellular K\(^+\) across the plasma membrane to provide energy for transport of various metabolites taking part in cell functions. Decreased activity of Na\(^+\), K\(^+\)-ATPase can lead to a decline in sodium efflux, which cause Na\(^+\) and water retention thereby altering the membrane permeability (Kako et al., 1988) that may lead to severe consequences. Na\(^+\), K\(^+\)-ATPase has been known to be a
good target of free radical induced membrane damage (Mense et al., 1997). Ca\textsuperscript{2+}-ATPase and Mg\textsuperscript{2+}-ATPase are membrane bound enzymes that catalyses the active transport of Ca\textsuperscript{2+} and Mg\textsuperscript{2+} across the cell membrane to maintain low intracellular Ca\textsuperscript{2+} and Mg\textsuperscript{2+} content. The intracellular concentration of calcium regulates the activity of the Mg\textsuperscript{2+}- and Na\textsuperscript{+}, K\textsuperscript{+}-ATPases. Disturbances in the function of Ca\textsuperscript{2+} ATPase activity thus alter the ion balance in the cells that leads to loss of cell function. The pumps are thus essential for the regulation of cell volume, uptake of nutrients, cell growth and differentiation and are critical for the normal functioning of excitable and non-excitatable tissues. Lipid peroxidation induced by free radicals has been shown to inactivate Na\textsuperscript{+}, K\textsuperscript{+}-ATPase by particularly modifying the active site for binding of the substrates (Mishra et al., 1989). Membrane proteins that control ion gradients across organellar and plasma membranes are particularly susceptible to oxidation-induced changes. Reductions in the activities of the membrane bound ATPases have been reported during oxidative stress due to hydroperoxides and drugs in liver hepatocytes (Salvi et al., 2005). Bironaite and Ollinger (1997) have reported that peroxidation can influence the functions of Ca\textsuperscript{2+}-, Mg\textsuperscript{2+}-ATPases.

In the present study, prior administration of taurine significantly (p<0.01) maintained the activities of membrane-bound ATPases at near normal levels when compared to that of Group III animals. Qi et al., 1995 report that taurine has been found to restore depletion of membrane Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity due to ozone exposure or cholesterol enrichment. Taurine is believed to don dual roles, both as an antioxidant to prevent lipid peroxidation and as a membrane stabilizer to maintain the environment for Na\textsuperscript{+}, K\textsuperscript{+}-ATPase to function properly (Huxtable, 1992 & Timbrell et al., 1 1995). Toker, et al., 2006 demonstrated that taurine restores the activity of Na+ K+ ATPase activity that was depressed by peroxinitrite administration. In a study on the effect of taurine on RBC exposed to high glucose, Nandhini & Anuradha, 2003 showed that taurine elevated the membrane ion pump activity. Stimulation
of Na\textsuperscript{+}/K\textsuperscript{+}ATPase activity by taurine could produce a decrease in uptake of Ca\textsuperscript{2+} due to the decreased activity of Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger. Sebring and Huxtable, 1985 showed that taurine stimulates the pumping rate of Ca\textsuperscript{2+}-activated ATPase pump possibly by increasing the turnover rate of the pump secondary to a membrane modification. The membrane activities of Na\textsuperscript{+}, K(\textsuperscript{+})-ATPase, Mg\textsuperscript{2+}, Ca(2\textsuperscript{+})-ATPase, were determined in the liver and brain of Wistar rats under acute hypoxic hypoxia against the background of preventive taurine administration and it was shown that the hypoxia-induced reduction in the activities of the ATPases were restored by taurine treatment (Man'kovskaia et al., 1992). Di Leo et al., 2002 have shown that chronic administration of taurine ameliorates oxidative stress and Na\textsuperscript{+} K\textsuperscript{+} ATPase impairment in the retina of diabetic rats. These observations suggest that taurine treatment protects liver plasma membrane against oxidative damage caused by D-GalN, by acting as an antioxidant membrane stabilizer and thus restoring of Na\textsuperscript{+}, K\textsuperscript{2+}-ATPase activity.

4.10 Mineral metabolism

The levels of plasma sodium (Fig 4.10.1) were significantly (p<0.001) lowered and liver sodium (Fig 4.10.2) markedly (P<0.001) increased in Group III D-GalN intoxicated rats when compared to normal Group I rats. There was also a significant (p<0.001) rise in plasma potassium (Fig 4.10.3) and fall (p<0.001) in liver potassium (Fig 4.10.4) levels in Group III rats in comparison with Group I rats. Further a significant (p<0.001) rise in the content of liver Ca (Fig 4.10.5) and decline (p<0.001) in the extracellular Ca (Fig 4.10.6) content was observed in Group III D-GalN injected rats when compared to normal Group I rats. D-GalN intoxication causes, impairment in the membrane protein Na\textsuperscript{+}, K\textsuperscript{+}-ATPase function through membrane damage. Disability of the pump results in failure of the cell to actively drive out Na\textsuperscript{+} causing its accumulation in the liver cell. Also in an injured cell, the rise in intracellular sodium concentration will lead to enhanced Ca\textsuperscript{2+}influx and increased intracellular Ca\textsuperscript{2+} via
Na+ Ca\(^{2+}\) exchanger, contributing to various cell dysfunctions (Kim et al., 2000). Due to the D-GalN-induced Ca\(^{2+}\) influx there is a marked (p<0.001) reduction in the extracellular Ca\(^{2+}\) levels. Active calcium transport and resultant low intracellular calcium concentration are essential requirements for proper functioning of Na\(^+\)/K\(^+\) ATPase pump (James et al., 1989). Since sodium and calcium are thought to be competitive at a number of membrane sites, it seems likely that a high concentration of Ca\(^{2+}\) in D-GalN-intoxicated hepatic cells, would compete with sodium specific sites at the inner surface of the membrane (Schatzmann, 1974), and this may lead to decrease in sodium being actively pumped out. Also, failure of sodium pump by itself results in a depletion of plasma sodium, rise in liver sodium, rise in plasma potassium and fall in liver potassium concentration (Lingrel & Kuntzweiler, 1994) as observed in the present study. Similar results have been described in several studies that have shown that in D-GalN and other hepatotoxins-mediated liver injury an increase in intracellular calcium is a commonly reported feature (Ikejima et al., 1997). Kroener and Planker 1980 report that female rats treated with D-galactosamine showed elevated liver calcium and decreased potassium contents four and eight hours after drug administration. Liver mitochondrial dysfunction such as the dissipation of mitochondrial membrane potential, mitochondrial swelling due to intra-mitochondrial Ca\(^{2+}\) overload, accompanied by morphological changes in mitochondria and the hepatocytes are also extensively reported (Gao et al., 2006 and Tang et al., 2006).

Prior administration of taurine to group IV rats has resulted in correction of D-GalN-induced alterations in the liver and plasma concentrations of calcium, sodium, and potassium when compared to D-GalN-intoxicated Group III rats. Lipid peroxidation and imperfect glycoprotein synthesis-induced decrease in Na\(^+\), K\(^+\)-ATPase and damage to protein-lipid membrane bilayer are restored by taurine supplementation (Toker et al., 2005 & Di Leo et al., 2002). Further, taurine treatment is thought to correct anomalies in mineral homeostasis.
in the following way: an increase in the concentration of intracellular Na\(^+\) activates taurine/Na symport which effluxes excess Na\(^+\) in combination with taurine. Thus taurine prevents the sodium related rise in intracellular calcium by enhancing taurine-Na\(^+\) symport. Taurine restores the levels of intracellular Ca\(^{2+}\). Some studies suggest that the mechanism of protection against injury afforded by taurine most likely involves inactivation of Kupffer cells. Taurine is shown to stimulate glycine-gated chloride channels, leading to a decrease in the intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) in cultured Kupffer cells stimulated with D-GalN (Ikejima et al., 1997). Taurine also is reported to alter the properties of Ca\(^{2+}\) binding sites on membrane acidic phospholipids thereby modifying Ca\(^{2+}\) delivery to the channel. Taurine directly affects the hydrophilic site on the channel and modifies the kinetics of channel opening or closing (Sawamura et al., 1990). These polyvalent actions of taurine on Ca\(^{2+}\) movement protect the cells against Ca\(^{2+}\) overload and restore the levels of sodium, potassium and calcium to a normal steady state.

4.11 Mitochondrial function

Mitochondria are important sub-cellular double membrane-bound organelles involved in energy production. The inner membrane holds the enzymes of electron transport chain that perform the redox reactions of oxidative phophorylation which generate membrane potential across the membrane. The final acceptor of ETC catalyses the reaction between reducing equivalents and molecular oxygen forming water and superoxide free radical. Mitochondria thus are a source of ROS namely, superoxide radical, hydroxyl radical, and singlet oxygen and are highly susceptible to ROS induced oxidative damage (Moro et al., 2005). Several disease conditions have been shown to correlate with an increase in oxidative stress and loss of mitochondrial function (Kadenbach et al., 2009). Cells require a continuous supply of glucose for energy production. Loss of mitochondrial function is disastrous for the liver since
ATP derived from oxidative phosphorylation is needed to maintain cellular activity (Sammut et al., 1998). Oxidative stress-induced mitochondrial dysfunction causes depletion of energy source in the form of ATP (Liu et al., 2002). Loss of ATP alters cell function by interrupting ATP-dependent processes, primarily the \( \text{Na}^+/\text{K}^+ \) ATPase, whose failure disrupts ionic gradients and membrane potentials across the membranes. It is critical that the inner mitochondrial membrane maintains a permeability barrier to protons, crucial for membrane potential and pH gradient generation, required for ATP synthesis through oxidative phosphorylation. When the inner membrane intergrity is disrupted, as is the case in D-GalN-induced hepatotoxicity (Vieira et al., 2000; Angermüller et al., 1998), mitochondria become uncoupled, and thus, can neither synthesize ATP by oxidative phosphorylation nor separate cytosolic and mitochondrial pools of metabolites (Miyahara et al., 1982; Anandan et al., 1999). Also, futile hydrolysis of ATP derived from glycolysis occurs in uncoupled and defunct mitochondria, as reversal of the ATP synthase takes place in the absence of membrane potential. Thus the damage inflicted in mitochondria would result in the reduction of energy production in the cell which leads to cell death (Suliman et al., 2003).

4.11.1 Protein, mitochondrial and respiratory marker enzymes

Figures 4.11.1.1, 4.11.1.2, 4.11.1.3, 4.11.1.4 & 4.11.1.5 show the level of protein in mitochondria and activities of mitochondrial citric acid cycle enzymes, isocitrate dehydrogenase, succinate dehydrogenase, malate dehydrogenase and respiratory marker enzyme (NADH dehydrogenase). In rats induced with FHF by D-Galactosamine (Group III) there was significant reduction \( (p < 0.001) \) in the activities of TCA cycle enzymes, NADH dehydrogenase and protein when compared with (Group I) control rats. The toxic effect of D-GalN, connected with an insufficiency of UDP sugars, inhibits nucleic acid and protein synthesis which affect the integrity of cell membranes and organelles (Keppler & Decker,
Moreover, it is known that D-GalN increases free-radical production and sensitizes hepatocytes to pro-inflammatory cytokines (Siendones et al., 2005). The later then exacerbate the oxidative stress impairing mitochondrial function (Chojkier & Fierer, 1985; Angermuller et al., 1999; Quintero et al., 2002). D-GalN also inhibits the energy metabolism of hepatocytes (Andreani et al., 1982). The depression of TCA cycle enzyme activities and ETC function as seen in D-GalN toxicity causes the accumulation of NADH and NADPH in the cells (Kroger et al., 1983). Failure of oxidation of these molecules accelerates the inactivation of cytochrome-P450 and causes destruction of nucleus, mitochondria and endoplasmic reticulum (Sohal et al., 1990). The activities of mitochondrial enzymes rely on transport of substrates and lipoprotein interactions across the inner membrane. D-GalN may indirectly affect enzyme activities of the mitochondria by inflicting injury and altering the phospholipid bilayer membranes (Sire et al., 1983). Succinate dehydrogenase, an important enzyme of TCA cycle harbored in the inner membrane is directly associated with the electron transport chain due to its ability to transfer electrons to the respiratory chain (Singh et al., 1988). D-GalN-induced oxidative stress alters the membrane structure thus affecting the activity of enzymes like succinate dehydrogenase (Padma & Setty, 1999). Loss of membrane integrity may also cause seepage of mitochondrial enzymes into the circulation contributing to the decline in their activity in the mitochondria. Mitochondrial respiration and oxygen uptake are reduced in the presence of high intracellular calcium. Rise in intracellular calcium is induced by D-GalN through disturbances in transport of calcium and other ions by inactivating of membrane pumps like Ca$^{2+}$ ATPase and Na$^+$ K$^+$ ATPase membrane. ATP-dependent calcium uptake and calcium binding was increased. Dolara et al., (1973) suggest that taurine reacts with calcium on the mitochondrial outer membrane and/or intermembrane spaces, increasing mitochondrial calcium-binding capacity and thus affecting mitochondrial oxygen uptake and respiration. Further evidence of D-GalN-inflicted damage to liver mitochondria are the ultra
structural changes like swollen mitochondria, loss of crisate with high tendency of mitochondrial ballooning as reported by Devaki et al., 2009.

Prior administration of taurine to animals intoxicated with D-Galactosamine (Group IV) revealed significant ($p < 0.001$) elevation in the activities of the TCA cycle enzymes and total mitochondrial protein (Figs 4.11.1.1, 4.11.1.2, 4.11.1.3, 4.11.1.4 and 4.11.1.5) when compared to Group III D-GalN-intoxicated rats. This is in agreement with several published reports. This protective effect of taurine rendered to mitochondria may be probably due to its potency to counter the free-radical mediated damage caused by D-GalN intoxication. Few previous studies have shown that taurine has good antioxidant activity and improves liver function against several drug-induced hepatotoxocities in experimental animals. Current opinion on the progression of liver injury maintains that cell death is the most crucial step (Kaplowitz, 2000). In hepatocytes as well as other cell types, the mitochondrion may serve as the arbiter of cell survival, releasing ROS and proteins that regulate cell death (Newmeyer & Ferguson-Miller, 2003; Orrenius, 2004). Mitochondrial membrane potential and calcium homeostasis in mitochondria are crucial for cells to stay healthy. In several forms of liver injury the mitochondrial membrane potential and the sensitivity to mitochondrial swelling induced by Ca$^{2+}$ were reduced. Thus drugs that check dissipation of membrane potential and prevent the decline in sensitivity of mitochondrial swelling could protect liver mitochondria in hepatotoxicity (Wallace, 1999; Tang et al., 2005). Taurine by virtue of its influence on membrane potential and stabilization and Ca$^{2+}$ modulation is expected to have protective effect on D-GalN-induced hepatotoxicity.

4.11.2 Mitochondrial antioxidant system

Oxidative stress is associated with a variety of pathological conditions, including cancer, diabetes, certain neurodegenerative diseases, ischemic heart disease, heart failure, and aging.
It is now well established that a major target of oxidative damage is mitochondrial DNA (mtDNA), and oxidative mtDNA damage has been implicated as a causative factor in each of these conditions. In D-GalN-induced hepatic injury a series of metabolic derangements culminates in extensive oxidative stress. Mitochondria are both a target and source of ROS.

In the present study protective effect of taurine was examined against D-GalN-induced oxidative injury in liver mitochondria. Lipid peroxides, soluble non-protein antioxidant glutathione, antioxidant enzymes GPX, GST, SOD and CAT were measured to evaluate the protective effect. D-GalN intoxicated Group III rats showed a significant (p<0.001) elevation in LPO (Fig 4.11.2.1) levels and a significant (p<0.001) decline in GSH (Fig 4.11.2.2), GSH dependent enzymes GPX and GST (Fig 4.11.2.3 & Fig 4.11.2.4) and antiperoxidative enzymes SOD and CAT (Fig 4.11.2.5 & Fig 4.11.2.6) when compared to normal Group I rats. These results are in agreement with other published reports.

D-GalN has been proposed to be hepatotoxic due to its ability to destruct liver mitochondrial membranes possibly by a free radical mechanism (Hu & Chen 1992). Lipid peroxidation reactions have been linked with altered membrane structure and enzyme inactivation (Comporti, 1985). The highly significant elevation in lipid peroxides in mitochondria of Group III D-GalN-induced hepatitis rats suggests the enhanced susceptibility of the membranes. Significant increases in the levels of mitochondrial lipid peroxides after i.p. administration of D-GalN have already been reported (Padma & setty, 1997). Depletion of GSH results in enhanced lipid peroxidation (Younes & Soegers 1981), and excessive lipid peroxidation can cause increased GSH consumption (Comporti, 1985), as observed in the present study. Tappel, 1965, has reported that GSH protects the mitochondrial membrane from the damaging action of lipid peroxide. GPX offers protection to the mitochondrial membrane from peroxidative damage (Umalakshmi & Devaki, 1992). A decrease in the activity of GPX makes mitochondria susceptible to D-GalN-induced damage, which leads to
a change in mitochondrial composition and function. The present study also shows decreased GPX activity in Group III D-GalN toxic rats, which is in line with the report by Neihorster et al., 1992. GPX and the cellular NADPH-generating mechanism together form a system for removing hydroperoxides from the cell. GST, another scavenging enzyme, binds to many different lipophilic compounds (Seishi et al., 1982); so it would be expected to bind D-GalN and act as an enzyme for GSH conjugation reactions. The significant decrease in its activity noted in this study might have been due to the decreased availability of GSH. This is consistent with a reported study (Irita et al., 1994), which showed a reduction of GST activity in liver. These findings led to the conclusion that depletion of GSH and GSH-dependent enzyme systems may be directly related to the pathogenic mechanism of D-GalN hepatitis. Sathivel et al., (2008) report that D-GalN- intoxicated rats showed liver damage with acute aberrations in serum lipid profile, lipid peroxidation, hepatic protein thiols, antioxidant enzymes and tissue non-enzymatic anti-oxidants. D-GalN and other hepatotoxins cause liver mitochondrial dysfunction through the dissipation of mitochondrial membrane potential, mitochondrial swelling and intra-mitochondrial Ca$^{2+}$ overload, accompanied by morphological changes in mitochondria (Gao et al., 2006; Miyahara et al., 1982).

Supplementation with taurine in group IV rats has significantly (p<0.001) prevented D-GalN-induced deviations in the mitochondrial parameters measured to assess the antioxidant potential of taurine when compared to the Group III rats because of its antioxidant nature (Ramesh et al., 1992). Glutathione has a direct antioxidant function. It functions in association with GST & GPX, by reacting with superoxide radicals, peroxy radicals and singlet oxygen to detoxify them. Taurine administration resulted in the elevation of GSH level, which protects against oxidative damage by regulating the redox status of proteins in the cell membrane (Inoue et al., 1987). Also activities of GPX and GST were maintained at near normal levels. Several previous studies support the results of the present study (Erdem et
which predominantly show that taurine prevents various toxin-mediated hepatic injuries by reducing oxidative stress, enhancing mitochondrial function, and modulating cytoplasmic and mitochondrial Ca\(^{2+}\) homeostasis.

Several studies (Nandhini et al., 2005; Hansen et al., 2006) have demonstrated that especially high taurine concentrations are found in tissues with high oxidative activity, whereas lower concentrations are found in tissues with primarily anaerobic activity. Taurine has been especially shown to be localized in the mitochondria. One particular study (Li et al., 2009) presents data signifying the important role of taurine as mitochondrial matrix buffer for stabilizing the mitochondrial oxidation explaining the anti-oxidative role of taurine. By stabilizing the environment in the mitochondria, taurine will prevent leakage of the reactive compounds formed in the mitochondrial environment and thus indirectly act as an antioxidant.

**4.11.3 Mitochondrial membrane stabilization (membrane-bound ATPases)**

In the present study a significant (p<0.001) decrease was noticed in the activities of mitochondrial total ATPase (Fig 4.11.3.1) and Ca\(^{2+}\) ATPase (Fig 4.11.3.2) in D-GalN-intoxicated Group III rats when compared to normal Group I rats. Disturbances in the functioning of the mitochondrial membrane transporters precipitates gross variations in the ionic flux across mitochondrial inner membrane. This has particular significance with respect to Ca homeostasis. D-GalN toxicity in rats is reported to cause extensive oxidative membrane damage in mitochondria along with loss enzyme and protein function. Miyahara et al., (1982) report that in vivo and in vitro treatments with D-GalN induce marked disorganization of mitochondrial structures.
Mitochondria have been implicated in the maintenance of the Ca\(^{2+}\) homeostasis in cells, where control of Ca\(^{2+}\) levels plays a significant role in enzymatic regulation and energy production. Loss of activity of membrane transporters Na\(^+\) K\(^+\) ATPase and Ca\(^{2+}\) ATPase causes marked alterations in the ion transport and contributes to disturbances in Ca, Na and K levels. Impairment of Ca\(^{2+}\)-sequestering by mitochondria cause an increase in cytoplasmic Ca\(^{2+}\) which is considered to be a critical event in apoptosis. Although the mechanisms responsible for cell injury under these conditions are not clear, excessive free cytosolic Ca\(^{2+}\) may lead to uncoupling of mitochondrial oxidative phosphorylation with consequently decreased ATP synthesis. The resulting inactivity of ATP-dependent pumps would lead to membrane depolarisation and further cellular Ca\(^{2+}\) influx. In this context, removal of Ca\(^{2+}\) to the extracellular space and/or uptake into organelles, including mitochondria, work to restore the normal cytoplasmic Ca\(^{2+}\) concentration.

Supplementation with taurine in Group IV rats restored (p<0.001) the activities of the membrane bound ATPases in mitochondria. In hepatocyte mitochondria, taurine was shown to prevent lipid peroxidation and to protect mitochondrial membrane against the cytotoxicity of different compounds such as hydrazine, carbon tetrachloride, and 1,4-naphthoquinone. Taurine is present in high (mM) concentrations in liver. One of its possible functions concerns modulation of Ca\(^{2+}\) transport. In many tissues, taurine has been shown to have marked effects on the kinetics of Ca\(^{2+}\) movement across the membranes of both cellular and subcellular preparations including rat liver mitochondria. In rats, a reduction of liver taurine significantly increased the hepatotoxicity of carbon tetrachloride, whereas treatment with taurine protected the liver against carbon tetrachloride-induced lipid peroxidation and concomitantly reduced intracellular Ca\(^{2+}\) accumulation, suggesting a correlation between the effect of taurine on Ca\(^{2+}\) and its protective effect. Changes in intracellular sequestration of Ca\(^{2+}\) have been suggested to explain the protective effect of taurine on galactosamine-induced
hepatic necrosis. Since D-GalN intoxication causes oxidative stress and disturbances in calcium homeostasis among other derangements, the present results suggest that the protection afforded by taurine may in part be related to its effects on mitochondrial function and Ca^{2+} sequestration.
Fig 4.1.1 Level of aspartate amino transferase (AST), alanine amino transferase (ALT) and lactate dehydrogenase (LDH) in plasma of control and experimental rats

For AST, ALT and LDH, one unit is defined as µmol of pyruvate liberated/h/l

(A): Taurine, 100mg kg⁻¹ body wt day⁻¹, i.p. for 30 days

(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.

^p<0.001 significantly different compared with Group I control animals

^b p<0.001 significantly different compared with Group II taurine-administered animals

^c p<0.001 significantly different compared with Group III D-GalN-induced hepatic failure rats
Fig 4.1.2 Levels of alkaline phosphotase, acid phosphatase and $\gamma$-glutamyl transferase in plasma of control and experimental rats

For $\gamma$-glutamyl transferase one unit is equivalent to $\mu$g of nitroanilide liberated/min/l

(A): Taurine, 100mg kg$^{-1}$ body wt day$^{-1}$, i.p. for 30 days

(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; SPSS version 10.

*p<0.001 significantly different compared with Group I control animals

*b*p<0.001 significantly different compared with Group II taurine-administered animals

*c*p<0.001 significantly different compared with Group III D-GalN-induced hepatic failure rats
Plate 4.2.1 The architecture of normal liver tissue in control rats. Stained with haematoxylin-eosin; 20X magnification.

Plate 4.2.2 The liver tissue in rats administered with taurine indicating no significant changes in architecture in comparison to the normal condition. Stained with haematoxylin-eosin; 20X magnification.
Plate 4.2.3 The architecture of liver tissue in D-galactosamine-administered rats showing necrosis (green arrow) with inflammatory cells (yellow arrow). Stained with haematoxylin-eosin; 20X magnification.

Plate 4.2.4 The architecture of liver tissues in rats treated with taurine and D-galactosamine, showing marked reduction in necrosis (green arrow) and inflammatory cells (yellow arrow). Stained with haematoxylin-eosin; 20X magnification.
**Fig 4.3.1** Levels of bilirubin in serum of control and experimental groups of rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days

(B): Galactosamine, 500mg100g\(^{-1}\) body weight day\(^{-1}\), i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.

\(^a\)\(p<0.001\) significantly different compared with Group I control animals

\(^b\)\(p<0.001\) significantly different compared with Group II taurine-administered animals

\(^c\)\(p<0.001\) significantly different compared with Group III D-galN-induced hepatic failure rats

**Fig 4.3.2** Levels of prothrombin time of control and experimental groups of rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days

(B): Galactosamine, 500mg100g\(^{-1}\) body weight day\(^{-1}\), i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.

\(^a\)\(p<0.001\) significantly different compared with Group I control animals

\(^b\)\(p<0.001\) significantly different compared with Group II taurine-administered animals

\(^c\)\(p<0.01\) significantly different compared with Group III D-galN-induced hepatic failure rats
Fig 4.4.2 Levels of arginase in liver of control and experimental groups of rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days

(B): Galactosamine, 500mg100g\(^{-1}\) body weight day\(^{-1}\), i.p. for 2 days

Results are mean ± SD for 6 animals; One way ANOVA; SPSS version 10.

\(^a\)p<0.01 significantly different compared with Group I control animals

\(^b\)p<0.01 significantly different compared with Group II taurine-administered animals

\(^c\)p<0.05 significantly different compared with Group III D-galN-induced hepatic failure rats

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Fig 4.4.3 Levels of urea in serum of control and experimental groups of rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days

(B): Galactosamine, 500mg100g\(^{-1}\) body weight day\(^{-1}\), i.p. for 2 days

Results are mean ± SD for 6 animals; One way ANOVA; SPSS version 10.

\(^a\)p<0.001 significantly different compared with Group I control animals

\(^b\)p<0.001 significantly different compared with Group II taurine-administered animals

\(^c\)p<0.001 significantly different compared with Group III D-galN-induced hepatic failure rats
**Fig 4.5.1.1** Levels of glycogen in liver of control and experimental groups of rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days

(B): Galactosamine, 500mg100g\(^{-1}\) body weight day\(^{-1}\), i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; SPSS version 10.

\(^a\)\(p<0.001\) significantly different compared with Group I control animals

\(^b\)\(p<0.001\) significantly different compared with Group II taurine-administered animals

\(^c\)\(p<0.001\) significantly different compared with Group III D-galN-induced hepatic failure rats

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**Fig 4.5.1.2** Activity of glycogen phosphorylase in liver of control and experimental groups of rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days

(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; SPSS version 10.

\(^a\)\(p<0.001\) significantly different compared with Group I control animals

\(^b\)\(p<0.001\) significantly different compared with Group II taurine-administered animals

\(^c\)\(p<0.001\) significantly different compared with Group III D-galN-induced hepatic failure rats
Fig 4.5.2.1 Levels of glucose in blood of control and experimental groups of rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; SPSS version 10.
\( ^{a}p<0.001 \) significantly different compared with Group I control animals
\( ^{b}p<0.001 \) significantly different compared with Group II taurine-administered animals
\( ^{c}p<0.001 \) significantly different compared with Group III D-galN-induced hepatic failure rats

Fig 4.5.2.2 Activity of hexokinase in liver of control and experimental groups of rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; SPSS version 10.
\( ^{a}p<0.001 \) significantly different compared with Group I control animals
\( ^{b}p<0.001 \) significantly different compared with Group II taurine-administered animals
\( ^{c}p<0.01 \) significantly different compared with Group III D-galN-induced hepatic failure rats
Fig 4.5.2.3 Activity of glucose-6-phosphate dehydrogenase in liver of control and experimental rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.

\( ^a p<0.001 \) significantly different compared with Group I control animals

\( ^b p<0.001 \) significantly different compared with Group II taurine-administered animals

\( ^c p<0.001 \) significantly different compared with Group III D-galN-induced hepatic failure rat

Fig 4.5.2.4 Activity of glucose-6-phosphatase in liver of control and experimental groups of rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.

\( ^a p<0.001 \) significantly different compared with Group I control animals

\( ^b p<0.001 \) significantly different compared with Group II taurine-administered animals

\( ^c p<0.001 \) significantly different compared with Group III D-galN-induced hepatic failure rats
Fig 4.5.2.5 Activity of fructose 1,6 bis phosphatase in liver of control and experimental rats

(A): Taurine, 100mg kg$^{-1}$ body wt day$^{-1}$, i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; SPSS version 10.

$^a$p<0.001 significantly different compared with Group I control animals

$^b$p<0.001 significantly different compared with Group II taurine-administered animals

$^c$p<0.01 significantly different compared with Group III D-galN-induced hepatic failure rats

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Fig 4.6.1 Level of protein in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg$^{-1}$ body wt day$^{-1}$, i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; SPSS version 10.

$^a$p<0.01 significantly different compared with Group I control animals

$^b$p<0.01 significantly different compared with Group II taurine-administered animals

$^c$p<0.05 significantly different compared with Group III D-galN-induced hepatic failure rats
Fig 4.6.2 Level of protein in liver tissue of control and experimental groups of rats

(A): Taurine, 100mg kg⁻¹ body wt day⁻¹, i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.

a p<0.01 significantly different compared with Group I control animals
b p<0.01 significantly different compared with Group II taurine-administered animals
c p<0.05 significantly different compared with Group III D-galN-induced hepatic failure rats

Fig 4.6.3 Levels of hexose in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg⁻¹ body wt day⁻¹, i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.

a p<0.001 significantly different compared with Group I control animals
b p<0.001 significantly different compared with Group II taurine-administered animals
c p<0.001 significantly different compared with Group III D-galN-induced hepatic failure rats
Fig 4.6.4 Levels of hexose in liver tissue of control and experimental groups of rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; One way ANOVA; SPSS version 10.

\(^a_p<0.001\) significantly different compared with Group I control animals
\(^b_p<0.001\) significantly different compared with Group II taurine-administered animals
\(^c_p<0.001\) significantly different compared with Group III D-galN-induced hepatic failure rats

Fig 4.6.5 Levels of hexoseamine in liver tissue of control and experimental rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; One way ANOVA; SPSS version 10.

\(^a_p<0.001\) significantly different compared with Group I control animals
\(^b_p<0.001\) significantly different compared with Group II taurine-administered animals
\(^c_p<0.001\) significantly different compared with Group III D-galN-induced hepatic failure rats
Fig 4.6.6 Levels of sialic acid in plasma of control and experimental group of rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; SPSS version 10.

\(^{a}p<0.001\) significantly different compared with Group I control animals

\(^{b}p<0.001\) significantly different compared with Group II taurine-administered animals

\(^{c}p<0.001\) significantly different compared with Group III D-galN-induced hepatic failure rats

Fig 4.6.7 Levels of sialic acid in liver tissue of control and experimental group of rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; SPSS version 10.

\(^{a}p<0.001\) significantly different compared with Group I control animals

\(^{b}p<0.001\) significantly different compared with Group II taurine-administered animals

\(^{c}p<0.001\) significantly different compared with Group III D-galN-induced hepatic failure rats
**Fig 4.7.1.1 Level of total cholesterol in plasma of control and experimental groups of rats**

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days

(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.

\(^a\)\(p<0.001\) significantly different compared with Group I control animals

\(^b\)\(p<0.001\) significantly different compared with Group II taurine-administered animals

\(^c\)\(p<0.01\) significantly different compared with Group III D-galN-induced hepatic failure rats

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**Fig 4.7.1.2 Level of VLDL-cholesterol in plasma of control and experimental rats**

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days

(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.

\(^a\)\(p<0.001\) significantly different compared with Group I control animals

\(^b\)\(p<0.001\) significantly different compared with Group II taurine-administered animals

\(^c\)\(p<0.001\) significantly different compared with Group III D-galN-induced hepatic failure rats
Fig 4.7.1.3 Level of LDL-cholesterol in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg⁻¹ body wt day⁻¹, i.p. for 30 days

(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.

a \( p < 0.001 \) significantly different compared with Group I control animals

b \( p < 0.001 \) significantly different compared with Group II taurine-administered animals

c \( p < 0.001 \) significantly different compared with Group III D-galN-induced hepatic failure rats

Fig 4.7.1.4 Level of HDL-cholesterol in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg⁻¹ body wt day⁻¹, i.p. for 30 days

(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.

a \( p < 0.001 \) significantly different compared with Group I control animals

b \( p < 0.001 \) significantly different compared with Group II taurine-administered animals

c \( p < 0.001 \) significantly different compared with Group III D-galN-induced hepatic failure rats
**Fig 4.7.1.5** Level of total cholesterol in liver of control and experimental groups of rats

(A): Taurine, 100mg kg⁻¹ body wt day⁻¹, i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; SPSS version 10.

\(^a\)\(p<0.001\) significantly different compared with Group I control animals

\(^b\)\(p<0.001\) significantly different compared with Group II taurine-administered animals

\(^c\)\(p<0.01\) significantly different compared with Group III D-galN-induced hepatic failure rats

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**Fig 4.7.2.1** Level of triglycerides in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg⁻¹ body wt day⁻¹, i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; SPSS version 10.

\(^a\)\(p<0.001\) significantly different compared with Group I control animals

\(^b\)\(p<0.001\) significantly different compared with Group II taurine-administered animals

\(^c\)\(p<0.001\) significantly different compared with Group III D-galN-induced hepatic failure rats
Fig 4.7.2.2 Level of triglycerides in liver tissue of control and experimental groups of rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; SPSS version 10.

\(^a\)\(p<0.001\) significantly different compared with Group I control animals

\(^b\)\(p<0.001\) significantly different compared with Group II taurine-administered animals

\(^c\)\(p<0.001\) significantly different compared with Group III D-galN-induced hepatic failure rats

Fig 4.7.2.3 Level of free fatty acids in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; SPSS version 10.

\(^a\)\(p<0.001\) significantly different compared with Group I control animals

\(^b\)\(p<0.001\) significantly different compared with Group II taurine-administered animals

\(^c\)\(p<0.001\) significantly different compared with Group III D-galN-induced hepatic failure rats
Fig 4.7.2.4 Level of free fatty acids in liver tissue of control and experimental groups of rats
(A): Taurine, 100mg kg⁻¹ body wt day⁻¹, i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days
Results are mean ± SD for 6 animals; Oneway ANOVA; SPSS version 10.
  "p<0.001 significantly different compared with Group I control animals
  "p<0.001 significantly different compared with Group II taurine-administered animals
  "p<0.001 significantly different compared with Group III D-galN-induced hepatic failure rats

Fig 4.7.2.5 Activity of triglyceride lipase in liver of control and experimental groups of rats
(A): Taurine, 100mg kg⁻¹ body wt day⁻¹, i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days
Results are mean ± SD for 6 animals; Oneway ANOVA; SPSS version 10.
  "p<0.001 significantly different compared with Group I control animals
  "p<0.001 significantly different compared with Group II taurine-administered animals
  "p<0.001 significantly different compared with Group III D-galN-induced hepatic failure rats
Fig 4.7.3.1 Level of phospholipids in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg$^{-1}$ body wt day$^{-1}$, i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; SPSS version 10.

$^a p<0.01$ significantly different compared with Group I control animals

$^b p<0.001$ significantly different compared with Group II taurine-administered animals

$^c p<0.01$ significantly different compared with Group III D-galN-induced hepatic failure rats

Fig 4.7.3.2 Level of phospholipids in liver tissue of control and experimental groups of rats

(A): Taurine, 100mg kg$^{-1}$ body wt day$^{-1}$, i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; SPSS version 10.

$^a p<0.001$ significantly different compared with Group I control animals

$^b p<0.001$ significantly different compared with Group II taurine-administered animals

$^c p<0.001$ significantly different compared with Group III D-galN-induced hepatic failure rats
Fig 4.7.4.1 Levels of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in liver tissue of control and experimental groups of rats

(A): Taurine, 100mg kg⁻¹ body wt day⁻¹, i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; One way ANOVA; SPSS version 10.

*<p>0.001 significantly different compared with Group I control animals

*b<p>0.01 significantly different compared with Group II taurine-administered animals

'p<0.01 significantly different compared with Group III galactosamine-induced hepatic failure rats

*d<p>0.05 significantly different compared with Group I control animals

'p<0.05 significantly different compared with Group II taurine-administered animals

'*p<0.05 significantly different compared with Group III galactosamine-induced hepatic failure rats

**p<0.01 significantly different compared with Group I control animals
Fig 4.7.4.2 Levels of n6 and n3 polyunsaturated fatty acids in liver tissue of experimental of rats
(A): Taurine, 100mg kg⁻¹ body wt day⁻¹, i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days
Results are mean ± SD for 6 animals; One way ANOVA; SPSS version 10.

*a p<0.05 significantly different compared with Group I control animals

*b p<0.01 significantly different compared with Group II taurine-administered animals

*c p<0.05 significantly different compared with Group III galactosamine-induced hepatic failure rats

Fig 4.7.4.3 Ratio of n6 and n3 polyunsaturated fatty acids in liver tissue of rats
(A): Taurine, 100mg kg⁻¹ body wt day⁻¹, i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days
Results are mean ± SD for 6 animals; One way ANOVA; SPSS version 10.

* p<0.05 significantly different compared with Group II taurine-administered animals
Fig 4.8.1.1 Level of lipid peroxides in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg⁻¹ body wt day⁻¹, i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; SPSS version 10.

*p<0.001 significantly different compared with Group I control animals

Ap<0.001 significantly different compared with Group II taurine-administered animals

Ap<0.001 significantly different compared with Group III D-galN-induced hepatic failure rats

Fig 4.8.2.1 Level of reduced glutathione (GSH) in liver tissue of control and experimental rats

(A): Taurine, 100mg kg⁻¹ body wt day⁻¹, i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; SPSS version 10.

*p<0.001 significantly different compared with Group I control animals

Ap<0.001 significantly different compared with Group II taurine-administered animals

Ap<0.001 significantly different compared with Group III D-galN-induced hepatic failure rats
**Fig 4.8.3.1. Activity of glutathione peroxidase in liver tissue of control and experimental rats**

(A): Taurine, 100mg kg$^{-1}$ body wt day$^{-1}$, i.p. for 30 days

(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.

$^a$p<0.001 significantly different compared with Group I control animals

$^b$p<0.001 significantly different compared with Group II taurine-administered animals

$^c$p<0.001 significantly different compared with Group III D-galN-induced hepatic failure rats

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**Fig 4.8.3.2 Activity of glutathione-S-transferase in liver tissue of control and experimental rats**

(A): Taurine, 100mg kg$^{-1}$ body wt day$^{-1}$, i.p. for 30 days

(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.

$^a$p<0.001 significantly different compared with Group I control animals

$^b$p<0.001 significantly different compared with Group II taurine-administered animals

$^c$p<0.001 significantly different compared with Group III D-galN-induced hepatic failure rats
Fig 4.8.4.1 Activity of catalase (CAT) in liver tissue of control and experimental groups of rats

(A): Taurine, 100mg kg$^{-1}$ body wt day$^{-1}$, i.p. for 30 days

(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.

$^a p<0.001$ significantly different compared with Group I control animals

$^b p<0.001$ significantly different compared with Group II taurine-administered animals

$^c p<0.001$ significantly different compared with Group III D-galN-induced hepatic failure rats

Fig 4.8.4.2 Activity of superoxide dismutase in liver tissue of control and experimental rats

Unit: One unit is the amount of protein required to give 50% inhibition of adrenaline autoxidation.

(A): Taurine, 100mg kg$^{-1}$ body wt day$^{-1}$, i.p. for 30 days

(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.

$^a p<0.001$ significantly different compared with Group I control animals

$^b p<0.001$ significantly different compared with Group II taurine-administered animals

$^c p<0.001$ significantly different compared with Group III D-galN-induced hepatic failure rats
Fig 4.9.1 Activity of Total ATPase in liver tissue of control and experimental groups of rats

(A): Taurine, 100mg kg⁻¹ body wt day⁻¹, i.p. for 30 days

(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.

\( ^a p<0.001 \) significantly different compared with Group I control animals

\( ^b p<0.001 \) significantly different compared with Group II taurine-administered animals

\( ^c p<0.001 \) significantly different compared with Group III D-galN-induced hepatic failure rats

Fig 4.9.2 Activity of Na⁺⁺, K⁺⁺-ATPase in liver tissue of control and experimental groups of rats

(A): Taurine, 100mg kg⁻¹ body wt day⁻¹, i.p. for 30 days

(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.

\( ^a p<0.001 \) significantly different compared with Group I control animals

\( ^b p<0.001 \) significantly different compared with Group II taurine-administered animals

\( ^c p<0.001 \) significantly different compared with Group III D-galN-induced hepatic failure rats
Fig 4.10.1 Levels of sodium in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days
(B): Galactosamine, 500mg 100g\(^{-1}\) body weight day\(^{-1}\), i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.

\( ^a p < 0.001 \) significantly different compared with Group I control animals

\( ^b p < 0.001 \) significantly different compared with Group II taurine-administered animals

\( ^c p < 0.001 \) significantly different compared with Group III GalN-induced hepatic failure rats

Fig 4.10.2 Levels of sodium in liver of control and experimental groups of rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days
(B): Galactosamine, 500mg 100g\(^{-1}\) body weight day\(^{-1}\), i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.

\( ^a p < 0.001 \) significantly different compared with Group I control animals

\( ^b p < 0.001 \) significantly different compared with Group II taurine-administered animals

\( ^c p < 0.001 \) significantly different compared with Group III GalN-induced hepatic failure rats
Fig 4.10.3 Levels of potassium in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days
(B): Galactosamine, 500mg 100g\(^{-1}\) body weight day\(^{-1}\), i.p. for 2 days

Results are mean ± SD for 6 animals; One way ANOVA; SPSS version 10.

\(^a\)\(p<0.001\) significantly different compared with Group I control animals

\(^b\)\(p<0.001\) significantly different compared with Group II taurine-administered animals

\(^c\)\(p<0.001\) significantly different compared with Group III GalN-induced hepatic failure rats

Fig 4.10.4 Levels of potassium in liver of control and experimental groups of rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days
(B): Galactosamine, 500mg 100g\(^{-1}\) body weight day\(^{-1}\), i.p. for 2 days

Results are mean ± SD for 6 animals; One way ANOVA; SPSS version 10.

\(^a\)\(p<0.001\) significantly different compared with Group I control animals

\(^b\)\(p<0.001\) significantly different compared with Group II taurine-administered animals

\(^c\)\(p<0.001\) significantly different compared with Group III GalN-induced hepatic failure rats
Fig 4.10.5 Levels of calcium in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days
(B): Galactosamine, 500mg 100g\(^{-1}\) body weight day\(^{-1}\), i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; SPSS version 10.

\(^a_p<0.001\) significantly different compared with Group I control animals

\(^b_p<0.001\) significantly different compared with Group II taurine-administered animals

\(^c_p<0.001\) significantly different compared with Group III GalN-induced hepatic failure rats

Fig 4.10.6 Levels of calcium in liver of control and experimental groups of rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days
(B): Galactosamine, 500mg 100g\(^{-1}\) body weight day\(^{-1}\), i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; SPSS version 10.

\(^a_p<0.001\) significantly different compared with Group I control animals

\(^b_p<0.001\) significantly different compared with Group II taurine-administered animals

\(^c_p<0.001\) significantly different compared with Group III GalN-induced hepatic failure rats
Fig 4.11.1.1 Level of protein in liver mitochondria of control and experimental groups of rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; One way ANOVA; SPSS version 10.

\(^{a}p<0.001\) significantly different compared with Group I control animals

\(^{b}p<0.001\) significantly different compared with Group II taurine-administered animals

\(^{c}p<0.001\) significantly different compared with Group III galN-induced hepatic failure rats

Fig 4.11.1.2 Activity of isocitrate dehydrogenase in liver mitochondria of rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; One way ANOVA; SPSS version 10.

\(^{a}p<0.001\) significantly different compared with Group I control animals

\(^{b}p<0.001\) significantly different compared with Group II taurine-administered animals

\(^{c}p<0.001\) significantly different compared with Group III galN-induced hepatic failure rats
Fig 4.11.1.3 Activity of succinate dehydrogenase in liver mitochondria of rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days

(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.

\(^a\) p<0.001 significantly different compared with Group I control animals

\(^b\) p<0.001 significantly different compared with Group II taurine-administered animals

\(^c\) p<0.001 significantly different compared with Group III GalN-induced hepatic failure rats

Fig 4.11.1.4 Activity of malate dehydrogenase in liver mitochondria of rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days

(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.

\(^a\) p<0.001 significantly different compared with Group I control animals

\(^b\) p<0.001 significantly different compared with Group II taurine-administered animals

\(^c\) p<0.001 significantly different compared with Group III GalN-induced hepatic failure rats
Fig 4.11.1.5  Activity of NADH dehydrogenase in liver mitochondria of control and experimental rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

\( ^a p < 0.001 \) significantly different compared with Group I control animals
\( ^b p < 0.001 \) significantly different compared with Group II taurine-administered animals
\( ^c p < 0.001 \) significantly different compared with Group III D-galN-induced hepatic failure rats
\( ^d p < 0.01 \) significantly different compared with Group II taurine-administered animals
\( ^e p < 0.05 \) significantly different compared with Group I control animals

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Fig 4.11.2.1  Level of lipid peroxides in liver mitochondria of control and experimental rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day), i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.
\( ^a p < 0.001 \) significantly different compared with Group I control animals
\( ^b p < 0.001 \) significantly different compared with Group II taurine-administered animals
\( ^c p < 0.001 \) significantly different compared with Group III GalN-induced hepatic failure rats
\( ^d p < 0.05 \) significantly different compared with Group I control animals
Fig 4.11.2.2

(A): Taurine, 100mg kg⁻¹ body wt day⁻¹, i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day, i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.

*p < 0.001 significantly different compared with Group I control animals

B *p < 0.001 significantly different compared with Group II taurine-administered animals

*C *p < 0.001 significantly different compared with Group III GalN-induced hepatic failure rats

D *p < 0.01 significantly different compared with Group II taurine-administered animals

Fig 4.11.2.3 Activity of glutathione peroxidase (GPx) in liver mitochondria of rats

(A): Taurine, 100mg kg⁻¹ body wt day⁻¹, i.p. for 30 days
(B): Galactosamine, 500mg/100g body weight/day, i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.

*p < 0.001 significantly different compared with Group I control animals

B *p < 0.001 significantly different compared with Group II taurine-administered animals

C *p < 0.001 significantly different compared with Group III GalN-induced hepatic failure rats

D *p < 0.01 significantly different compared with Group II taurine-administered animals
Fig 4.11.2.4 Activity of glutathione-S-transferase (GST) in liver mitochondria of rats

(A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days

(B): Galactosamine, 500mg/100g body weight/day, i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.

\(^a\)\(p<0.001\) significantly different compared with Group I control animals

\(^b\)\(p<0.001\) significantly different compared with Group II taurine-administered animals

\(^c\)\(p<0.001\) significantly different compared with Group III GalN-induced hepatic failure rats

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Fig 4.11.2.5 Activity of superoxide dismutase (SOD) in liver mitochondria of rats

Unit: One unit of the SOD activity is the amount of protein required to give 50% inhibition of adrenaline autoxidation. (A): Taurine, 100mg kg\(^{-1}\) body wt day\(^{-1}\), i.p. for 30 days

(B): Galactosamine, 500mg/100g body weight/day, i.p. for 2 days

Results are mean ± SD for 6 animals; One-way ANOVA; SPSS version 10.

\(^a\)\(p<0.001\) significantly different compared with Group I control animals

\(^b\)\(p<0.001\) significantly different compared with Group II taurine-administered animals

\(^c\)\(p<0.001\) significantly different compared with Group III GalN-induced hepatic failure rat
**Fig 4.11.2.6 Activity of catalase (CAT) in liver mitochondria of control and experimental rats**

(A): Taurine, 100mg kg$^{-1}$ body wt day$^{-1}$, i.p. for 30 days

(B): Galactosamine, 500mg100g$^{-1}$ body weight day$^{-1}$, i.p. for 2 days

Results are mean ± SD for 6 animals; One way ANOVA; SPSS version 10.

$^a p<0.001$ significantly different compared with Group I control animals

$^b p<0.001$ significantly different compared with Group II taurine-administered animals

$^c p<0.001$ significantly different compared with Group III GalN-induced hepatic failure rats

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**Fig 4.11.3.1 Total -ATPase in liver mitochondria of control and experimental groups of rats**

(A): Taurine, 100mg kg$^{-1}$ body wt day$^{-1}$, i.p. for 30 days

(B): Galactosamine, 500mg100g$^{-1}$ body weight day$^{-1}$, i.p. for 2 days

Results are mean ± SD for 6 animals; One way ANOVA; SPSS version 10.

$^a p<0.001$ significantly different compared with Group I control animals

$^b p<0.001$ significantly different compared with Group II taurine-administered animals

$^c p<0.001$ significantly different compared with Group III GalN-induced hepatic failure rats
Fig 4.11.3.2 Ca\textsuperscript{2+} -ATPase in liver mitochondria of control and experimental groups of rats

(A): Taurine, 100mg kg\textsuperscript{-1} body wt day\textsuperscript{-1}, i.p. for 30 days

(B): Galactosamine, 500mg100g\textsuperscript{-1} body weight day\textsuperscript{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; One way ANOVA; SPSS version 10.

\textsuperscript{a}\(p<0.001\) significantly different compared with Group I control animals

\textsuperscript{b}\(p<0.001\) significantly different compared with Group II taurine-administered animals

\textsuperscript{c}\(p<0.001\) significantly different compared with Group III GalN-induced hepatic failure rats