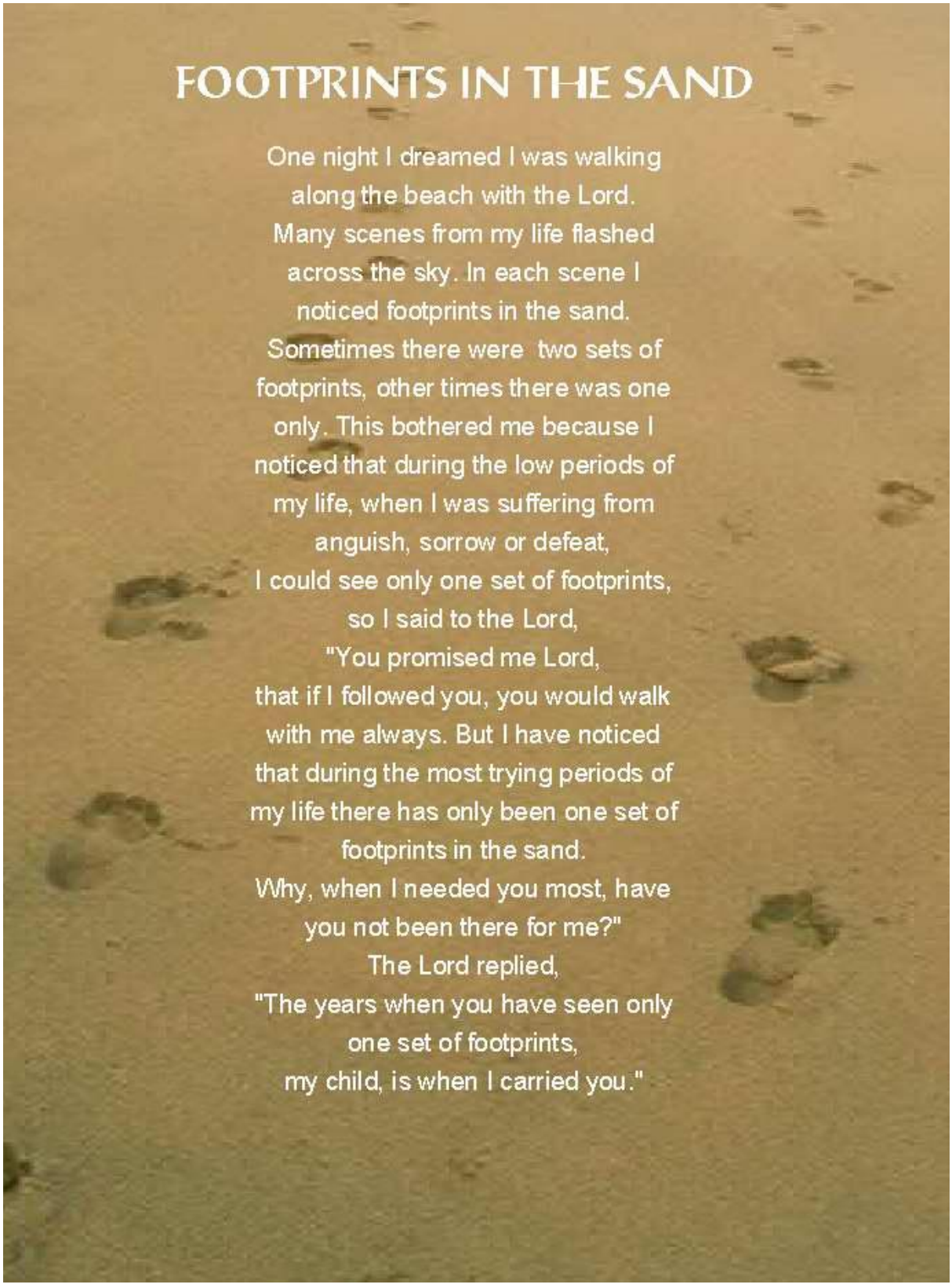
DEDICATED TO THE MEMORIES OF

MY DEAR MOTHER & FATHER


FOOTPRINTS IN THE SAND
One night I dreamed I was walking
along the beach with the Lord.
Many scenes from my life flashed
across the sky. In each scene I
noticed footprints in the sand.
Sometimes there were two sets of
footprints, other times there was one
only. This bothered me because I
noticed that during the low periods of
my life, when I was suffering from
anguish, sorrow or defeat,
I could see only one set of footprints,
so I said to the Lord,
"You promised me Lord,
that if I followed you, you would walk
with me always. But I have noticed
that during the most trying periods of
my life there has only been one set of
footprints in the sand.
Why, when I needed you most, have
you not been there for me?"
The Lord replied,
"The years when you have seen only
one set of footprints,
my child, is when I carried you."

COCHIN UNIVERSITY

CERTIFICATE

I, **Dr. K.Devadasan**, certify that the thesis entitled **"BIOCHEMICAL STUDIES ON THE PROTECTIVE EFFECT OF TAURINE ON EXPERIMENTALLY INDUCED FULMINAT HEPATIC FAILURE IN RATS"** submitted for the **Degree of Doctor of Philosophy** by **Ms. K.K.Asha** is an authentic record of research work carried out by her during the period from November 2004 to October 2009 under my guidance and supervision and that this has not formed the basis for the award of any degree, diploma, associate-ship, fellowship or other titles in this University or any other University or Institutions of higher learning.

Place: Cochin
Date: 23.06.2010

(Dr. K. Devadasan)
Former Director
CIFT, Cochin-682029

COCHIN UNIVERSITY

DECLARATION

I, **K.K.Asha** declare that the thesis entitled **"BIOCHEMICAL STUDIES ON THE PROTECTIVE EFFECT OF TAURINE ON EXPERIMENTALLY INDUCED FULMINAT HEPATIC FAILURE IN RATS"** submitted by me for the **Degree of Doctor of Philosophy** is the record of research work carried out by me during the period from November 2004 to October 2009 under the guidance of **Dr. K. Devadasan**, Former Director, Central Institute of Fisheries Technology, Cochin-682029, and that this has not formed the basis for the award of any degree, diploma, associate-ship, fellowship, titles in this or any other University or other similar Institutions of higher learning.

Place: Cochin
Date: 23.06.2010 **(K.K.Asha)**

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K.K.ASHA

CONTENTS

LIST OF ABBREVIATIONS

ADP	-	Adenosine -5-diphosphate
ALT	-	Alanine aminotransferase
ANSA	-	Aminonaphthosulfonic acid
APS	-	Ammonium per sulphate
AST	-	Aspartate aminotransferase
ATP	-	Adenosine triphosphate
BHT	-	3,5 dibutyl-4-hydroxy toluene
BSA	-	Bovine serum albumin
cAMP	-	Cyclic adenosine monophosphate
CAT	-	Catalase
CDNB	-	1-Chloro-2, 4-dinitrobenzene
CE	-	Cholesteryl esters
CHM	-	Chloroform, Heptane, Methanol
CPCSEA	-	Committee for the purpose of control and supervision of experiments on animals
DDC	-	Diethyldithiocarbomate
D-GalN	-	D-galactosamine
DHA	-	Docosahexaenoic acid
dl	-	Decilitre
DNPH	-	2,4 Dinitrophenyl hydrazine
DTNB	-	5,5'-Dithiobis(2-nitrobenzoic acid)
EPA	-	Eicosapentaenoic acid
EDTA.	-	Ethelene diamine tetraacetic acid
ER	-	Endoplasmic reticulum
FAME	-	Fatty acid methyl ester
$FeCl_3$	-	Ferric chloride
GABA	-	Gamma amino butyric acid
GGT	-	Gamma glutamyl transferase
FFA	-	Free fatty acid
GLUT4	-	Glucose transporter
GPx	-	Glutathione peroxidase
GSH	-	Reduced glutathione

GST	-	Glutathione-S-transferase
HDL	-	High density lipoprotein
HPLC	-	High performance liquid chromatography
h	-	Hours
i.p	-	Intra peritoneal
IAEC	-	Institutional Animal Ethics Committee
IU	-	International unit
LDH	-	Lactate dehydrogenase
LDL	-	Low-density lipoprotein
$LOO^{\bullet}$	-	Lipid peroxy radical
LPO	-	Lipid peroxides
MDA	-	Malondialdehyde
NAD+	-	Nicotinamide adenine dinucleotide
NADH	-	Reduced nicotinamide adenine dinucleotide
NADP	-	Nicotinamide adenine dinucleotide phosphate
OD	-	Optical density
OTC	-	Ornithine transcabamoylase decarboxylase
$OH^{\bullet}$	-	Hydroxyl radical
OPA	-	O-Phthaladehyde
PDH	-	Pyruvate dehydrogenase
PE	-	Petroleum ether
PEG	-	Polyethylenglycol
Pi	-	Inorganic phosphorus
PKA	-	Protein kinase A
PL	-	Phospholipids
PUFA	-	Poly unsaturated fatty acids
ROS	-	Reactive oxygen species
rpm	-	Revolution per minute
SD	-	Standard deviation
SDS-PAGE	-	Sodium dodecil sulphate-polyacrylamide gel electrophoresis
SOD	-	Superoxide dismutase
TBA	-	Thiobarbituric acid
TBARS	-	Thiobarbituric acid reactive substances

TCA cycle	-	Tri carboxylic acid cycle
TCA	-	Trichloroacetic acid
TEMED	-	N,N,N^1,N^1 -Tetra methyl ethylene diamine
TEP	-	Tetraethoxy propane
TG	-	Triglyceride
VLDL	-	Very low-density lipoprotein

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Abstract

Fulminant hepatic failure (FHF) is a dramatic and challenging syndrome in clinical medicine. Although an uncommon disorder, it is usually fatal and occurs in previously healthy person. While the causes of FHF remain unclear, viral hepatitis and drug-induced liver injury account for the majority of cases. Hepatitis E causes large-scale epidemics of hepatitis in the Indian subcontinent, involving hundreds of thousands of cases with high mortality. FHF is associated with several clinical features like jaundice, shrunken liver, easy bruising, low levels of serum proteins, fatigue, multi-organ failure etc and metabolic derangements like hypoglycemia, hyperlipidemia, hyponatremia, defective protein synthesis, reduced energy production, decreased rate of urea production etc. These disturbances are predominantly attributed to oxidative stress, membrane destabilization and osmolytic imbalances. The options available for these patients are quite minimal with liver transplantation being one of them. But the procedure is ridden with issues causing it to find less favor among the patients and the caregivers. Use of hepatoprotective and cytoprotective drugs, is being considered to be a more acceptable alternative as a strategy to enhance liver regeneration. In this regard use of taurine a naturally occurring amino acid that plays a crucial role in many physiological processes would prove to be effective. In the present study, hepatoprotective effect of taurine on a rat model of induced FHF was studied. Taurine supplementation has effectively counteracted the metabolic and structural aberrations in the liver caused by D-galactosamine intoxication.

INTRODUCTION

1. INTRODUCTION

Fulminant hepatic failure (FHF) is a rare, potentially devastating syndrome characterized by the abrupt collapse of liver function and hepatic encephalopathy. It is a serious and fatal disease which causes significant morbidity and mortality (Fernandez *et al.,* 2003). It is a condition in which the rapid deterioration of liver function results in alteration in the mental status of a previously healthy individual. Fulminant hepatic failure is one of the leading causes of death in hospitalized children in India (Alam *et al.,* 2009). The condition is particularly distressing as it occurs acutely, in previously healthy children and progresses rapidly inspite of all modern treatment. FHF, characterized by severe metabolic derangements, neurologic complications and, ultimately, multiorgan failure and in many cases by death, is seen to be a syndrome that may result from numerous causes.

Etiology*:* FHF can result from diverse etiological agents. Of these, hepatitis viruses, acetaminophen overdose and idiosyncratic drug reactions account for the bulk of cases, drugs, poisons, toxins and metabolic disorders being relatively less common causes (Gotthardt *et al.,* 2007). Metabolic and vascular liver diseases, liver diseases unique in pregnancy and a number of miscellaneous liver diseases cause a small number of the remaining cases. Viral hepatfitis is nearly the sole causative agent of FHF in the Indian subcontinent, the most important cause in Europe and the United States, and comes next only to acetaminophen hepatotoxicity as a cause of FHF in the United Kingdom (McCrudden *et al.,* 2000, Kwo *et al.,* 1995).

Sex & Age: Distribution of FHF is equal among males and females. Children and adults of all ages may develop FHF (Arora *et al.,* 1996).

Incidence*:* FHF often affects young people and results in a very high mortality. Hepatitis E causes large-scale epidemics of hepatitis leading to FHF in the Indian subcontinent (Acharya *et al.,* 1996), affecting hundreds of thousands of people, high mortality (Khan *et al.,* 2006). In

the US the incidence of FHF is low with approximately 2000 cases occurring annually (Hoofnagle *et al.,* 1996). Drug-related hepatotoxicity comprises more than 50% of ALF cases, including acetaminophen toxicity (42%) and idiosyncratic drug reactions (12%). Nearly 15% of cases remain of indeterminate etiology.

Survival rate*:* Despite aggressive treatment, many patients die from FHF. Prior to liver transplantation for FHF, the mortality rate was generally greater than 90% (Atillasoy & Berk 1995). However, with improved intensive care, the prognosis is much better now than in the past (Ostapowicz *et al.,* 2002). The development of liver support systems provides some promise for this particular circumstance but has no impact on survival.

Mortality and Morbidity FHF is fatal for most affected children below 10 years and adults above 40 years of age. Several factors contribute to morbidity and mortality. The etiologic factor leading to hepatic failure and the development of complications plays an important role. In general, the best prognoses occur in the absence of complications. Cerebral edema, renal failure, adult respiratory distress syndrome, bleeding, and sepsis pose challenges that reduce the probability of survival (Shakil *et al.,* 2000). Early recognition and hospitalization is the most important factor in reduction of mortality from FHF. In the past three decades, improved intensive care has increased mean survival from 15% to 50% in certain patient groups by providing metabolic support and management of specific, frequent, and potentially fatal complications (Ericzon *et al.,* 2001).

Pathogenesis: The pathogenesis of FHF usually begins with exposure of a susceptible person to an agent capable of producing severe hepatic injury. Viral agents cause damage to hepatocytes either by direct cytotoxic effect or as a result of hyperimmune response (Seneviratne *et al.,* 2006). Also hepatotoxic metabolites, which accumulate as a result of errors in metabolism or of taking drugs, cause injury to the hepatocytes resulting in accumulation of neurotoxic substances (Sturgill and Lambert, 1997).

Management: FHF remains a vexing and lethal clinical problem for liver specialists. Good intensive care is critical for patient survival. There are no specific therapies for FHF; medical management at the present time requires a multidisciplinary approach and must focus on anticipating, preventing, and rapidly identifying and treating complications that may affect every major organ system (Hoofnagle *et al.,* 1996). Monitoring and early treatment of infections, hemodynamic abnormalities, and brain edema is critical to patient survival. Liver transplantation is a definitive therapeutic option for situations in which spontaneous recovery appears unlikely (Hadem *et al.,* 2008). The diverse etiologies and difficulty in predicting which patients will recover spontaneously from those who will die without timely liver transplantation contribute to the complexity of this condition (Gotthardt *et al.,* 2007). While intensive care is sufficient therapy in some patients (Group I), those with irreparable hepatic damage (Group III) can only survive if transplanted. In intermediate cases (Group II), the liver retains the potential to regenerate if the patient receives hepatic functional support (Atillasoy & Berk 1995). Liver transplantation has dramatically improved the chances of survival for patients with FHF, with current survival rates in the 55% to 75% range (Farmer *et al.,* 2003). However the procedure is ridden with several issues that makes liver transplantation a less favorable alternative. The continuing challenge for the transplant team is to identify critical patients who would not survive without transplantation and allocate available donor organs to them. At the same time it is important to provide liver transplantation in a timely fashion to ensure the best chance of post-transplantation recovery. Factors that are valuable in assessing the likelihood of spontaneous recovery are features such as patient's age, cause of FHF and dynamic features including severity of encephalopathy, prothrombin time, and serum bilirubin. However, the accuracy of these predictive indices decreases when they are applied to different populations, probably because of regional differences in etiology. Early prediction and timely availability of donor livers are essential

for a successful outcome. A donor shortage, however, continues to pose problems for hepatologists (Wiesner, 2005). Another impediment to successful revival of patients suffering from FHF is that liver transplantation needs to be individualized for each patient, because recovery depends on the cause of the hepatic failure. New therapeutic alternatives to liver transplantation are required. Effective liver support devices and hepatoprotective agents may greatly prolong survival to receive a donor liver, or alternatively to allow the native liver to regenerate (Lesnikov *et al.*, 2004). A better understanding of mechanism of liver cell death and multiorgan failure, and the development of strategies to accelerate and maximize hepatic regeneration, may allow a more targeted approach to therapy. Encouraging research is being carried out in identifying innovative approaches to management and therapy. These include clinical application of cytoprotective and hepatotrophic drugs or antiviral medications and artificial hepatic support systems. None of these are of proven benefit, but many are promising as a means to support the patient with FHF until spontaneous recovery occurs or a suitable liver is available for transplantation.

A wide range of chemical agents and plant extracts have been analyzed for possible hepatoprotective effect. Most of these are synthetic and many are known to produce undesirable side effects (Stickel *et al.*, 2000). Increasing attention is being given world wide on therapeutic effects of naturally occurring substances like vitamins, minerals, amino acids, small bioactive peptides commonly referred to as nutraceuticals. Taurine (2-aminoethanesulfonic acid), a non-protein sulfur containing amino acid, is the most abundant free amino acid and has been shown to play several essential roles in the human body. It is widely distributed in very high concentrations in brain, heart, liver, kidney, lens, and reproductive organs (Hayes, 1988). It is involved in various important biological and physiological functions, which include cell membrane stabilization, antioxidation,

detoxification, osmoregulation, neuromodulation and brain and retinal development (Kendler, 1989; Huxtable, 1992).

Fish is one of the best sources of taurine and for the same reason dietary fish can be a good nutritional supplement for ensuring adequate supply of taurine to the system (Gormley *et al.,* 2007). Our studies on nutritional benefits of dietary fish pointed to such an effect that led us to a more detailed investigation into the protective effect of taurine in hepatotoxicity. The results and observations made in this study forms the topic of this thesis. Earlier studies demonstrated that pathology develops if the animal is depleted of taurine stores either through a taurine deficient diet or use of taurine transport antagonists (Gupta & Kim, 2003). There is considerable evidence concerning the pharmacological significance of taurine in maintaining the integrity of organism (Chesney, 1995). Taurine is reported to stabilize membranes, inhibit oxidative stress and Kupffer cell activation, phenomena associated with most types of liver (Timbrell *et al.,* 1995). These effects may play an important role in taurine's expected hepatoprotective effects.

Intraperitoneal (i.p.) intoxication of rats by D-galactosamine (D-GalN) produces a reproducible experimental model of FHF resembling the clinical condition. Numerous research studies carry reports of D-GalN injection producing metabolic and histopathological alterations which are similar to FHF condition (Feng *et al.,* 2007; Takamura *et al.,* 2007; Wu *et al.,* 2009). They include rise in liver specific markers in blood, elevation of certain cytokines, prothrombin, bilirubin and extensively necrotic hepatic tissue (Hu *et al.,* 1992). As a standard technique D-GalN was used in this work to induce FHF symptoms in albino rats, to study the hepatoprotective effect if any, of taurine in FHF case.

1.1 Aim: To successfully induce and maintain animal model of FHF by i.p. injection of D-GalN and study the protective effect of taurine treatment.

Objectives:

1. To study the hepatoprotective effects of taurine in experimentally induced FHF by assaying the levels of serum diagnostic marker enzymes, prothrombin time, and bilirubin.
2. To study the histopathological pattern to confirm the protective action of taurine against-D-GalN-induced FHF in rats.
3. To determine the effect of taurine on protein and glycoprotein content of tissue and serum in the experimental model of FHF.
4. To study the effect of taurine on glucose metabolism in induced FHF.
5. To evaluate the effect of taurine on lipid metabolism in D-GalN-induced FHF in rats by determining the levels of various lipid components.
6. To study the effect of taurine on fatty acid profile in experimentally induced FHF in rats.
7. To study the anti-peroxidative effect of taurine on tissue antioxidant defense system in D-GalN-induced FHF in rats.
8. To determine the effect of taurine on mineral homeostasis and assess the membrane stabilizing action of taurine by assaying the activities of membrane bound ATPases.
9. To study the effect of taurine on mitochondrial function in experimentally induced FHF by determining the activities of TCA cycle enzymes and respiratory marker enzymes.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 Fulminant hepatic failure

Trey and Davidson introduced the term *fulminant hepatic failure* in 1970 to describe a "potentially reversible condition, the consequence of severe liver injury, with an onset of encephalopathy within two weeks of the appearance of jaundice and in the absence of pre-existing liver disease (Trey & Davidson 1970). The lack of prior liver disease is critical to the definition of FHF. Patients who suffer an acute deterioration of previously stable cirrhosis from alcohol or chronic hepatitis may have a life-threatening illness, but they do not have FHF. Unlike such patients with acute or chronic liver disease, patients with FHF have the potential to completely recover normal liver function, although this is far more likely with some causes of FHF than with others (Cillo *et al.*, 2004). Despite its relative rarity, FHF is important because it is associated with high mortality (Mohanty & Schiff 2009). However, the dismal prognosis associated with FHF appears to be improving, at least in the developed world. This trend probably points to the improvement in medical intensive care, and a change in the epidemiology of FHF with a shift from causes with a relatively poor predictability (e.g., hepatitis related causes) to those with a relatively good predictability (e.g. acetaminophen overdose) (Vaquero & Blei, 2003) .

2.1.1 Risk factors

A diverse array of insults can cause FHF. Liver failure due to viral attack is currently the most common cause of FHF in developing nations (Poddar *et al.,* 2002) while liver injury from drugs and toxins forms the largest cause in the US and the UK (Alam *et al.,* 2009). Older infants and children are more likely to develop FHF from viral hepatitis. The following is a brief classification of the causes of FHF. Selected etiologies of FHF are discussed below.

Viral infection: Hepatitis A, B & E and Herpes simplex virus

Drugs and toxins: Acetaminophen & other drugs, amanita phalloides, isoniazid, aflatoxin, halothane are associated with development of FHF.

Vascular problems: These causes include portal vein thrombosis (Murad *et al.,* 2006), hepatic artery thrombosis (Montalti *et al.,* 2005), ischemic hepatitis (Gibson & Dudley 1984), vascular shock (Morishita 1984) or tumor infiltration of the liver (Rowbotham *et al.,* 1998).

Metabolic causes: Causes in very young infants include neonatal iron storage disease (Barnard & Manci 1991), certain metabolic disorders such as tyrosinemia (Scriver *et al.,* 1967) and galactosemia (Suchy, 1996) peroxisomal diseases (Roels *et al.*,1991), and defects in respiratory chain (Goncalves *et al.,* 1995) and synthesis of bile acid (Lee *et al.,* 2001). Family screening is appropriate for many metabolic or genetic causes of FHF.

Indeterminate causes: Approximately 15-20% of adult cases and upto 50% of FHF in children cannot be attributed to a specific cause (Davern 2004).

2.1.1.1 Viruses

In parts of Asia and most of Africa, viruses represent the lead cause of FHF (Capocaccia and Angelico, 1991). In the past, viral hepatitis B (HBV) was one of the leading causes of FHF in the US, but its incidence has been declining. It is now a rare cause of FHF in the US, accounting for about 5% of FHF cases (Polson & Lee, 2005). *Acute viral hepatitis* is diffuse liver inflammation caused by specific hepatotropic viruses that have diverse modes of transmission and epidemiologies and each type shares clinical, biochemical, and morphologic features (Chu & Liaw, 1990). Acute infection tends to develop in predictable phases. Infection begins with an incubation period during which the virus multiplies and spreads without symptoms. The pre-icteric phase follows, producing nonspecific symptoms, such as profound anorexia, malaise, nausea and vomiting, and, often, fever or right upper quadrant

abdominal pain. During the icteric phase, the liver is usually enlarged and tender. Most cases resolve spontaneously, but some progress to chronic hepatitis. Occasionally, acute viral hepatitis progresses to fulminant hepatic failure (Vento *et al.,* 1998).

Etiology and Epidemiology: At least five specific viruses are known to cause hepatitis **(Table2.1.1.1)**.

Hepatitis A virus (HAV): HAV is a single-stranded RNA virus. It is the most common cause of acute viral hepatitis and is particularly common among children and young adults (Dmochowski, 1976). Infection with hepatitis A virus is usually transmitted by a fecal-oral route and thus may occur in areas of poor hygiene. Waterborne and food-borne epidemics occur, especially in underdeveloped countries. Eating contaminated raw shellfish is sometimes responsible.

Hepatitis B virus (HBV): HBV is the most thoroughly characterized and complex hepatitis virus. The infective particle consists of a viral core that contains circular double-stranded DNA and it replicates within the nuclei of infected hepatocytes (Jilbert 1992). HBV is the second most common cause of acute viral hepatitis (Joshi 1984). Routine screening of donor blood for hepatitis B surface antigen (HBsAg) has nearly eliminated the previously common post transfusion transmission, but transmission through needles shared by drug users remains common. Prevalence varies widely according to several factors, including geography (eg, < 0.5% in North America and northern Europe, > 10% in some regions of the Far East) (Dmochowski, 1976, Snyder & Pickering, 2004). Vertical transmission from mother to infant is common in China and other parts of Asia (WHO document, 1998).

Hepatitis C virus (HCV): HCV is a single-stranded RNA virus. Six major HCV subtypes exist with varying genotypes; these subtypes vary geographically and in virulence and response to therapy (Alter, 1997). Infection is most commonly transmitted through blood, primarily when parenteral drug users share needles (Ridzon, 1997) but also through tattoos

Table 2.1.1.1: Characteristics of Hepatitis Viruses

	Hepatitis A Virus	Hepatitis B Virus	Hepatitis C Virus	Hepatitis D Virus	Hepatitis E Virus
Nucleic acid	RNA	DNA	RNA	*	RNA
Major transmission	Fecal–oral	Blood	Blood	Needle	Water
Incubation(days)	15–	40–180	20–120	30–180	14–60
Epidemics	Yes	No	No	No	Yes
Chronicity	No	Yes	Yes	Yes	No
Liver cancer	No	Yes	Yes	Yes	No

*Incomplete RNA; requires presence of hepatitis B virus for replication.

(Haley and Fischer 2001). Up to 20% of patients with alcoholic liver disease harbor HCV (Coelho–Little, 1995); both HCV and alcohol act to exacerbate liver damage.

Hepatitis D virus (HDV): HDV, or delta agent, is a defective RNA virus that can replicate only in the presence of HBV (Smedile 1994). It occurs uncommonly as a co-infection with hepatitis B. Prevalence of HDV varies widely geographically, with endemic pockets in several countries (Jacobson 1995).

Hepatitis E virus (HEV): Hepatitis E virus (HEV) is associated with a high incidence of FHF in women who are pregnant in regions like Mexico and Central America, India and the subcontinent, and the Middle East (Navaneethan *et al.,* 2008). It is the most common cause of FHF in endemic regions of the world, with high mortality (25%) in pregnant women (Kumar *et al.,* 2004). HEV is an enterically transmitted RNA virus. Outbreaks of acute HEV infection, often waterborne and linked to fecal contamination of the water supply, have

occurred in China, India, Mexico, Pakistan, Peru, Russia, and central and northern Africa (Corwin, 1996).

2.1.1.2 Acetaminophen

Acetaminophen poisoning is the leading cause of FHF in the developed countries like US and the UK and is responsible for nearly 50% of all cases (Larson *et al.,* 2006). FHF due to acetaminophen poisoning may result from the intentional or unintentional overdosing. There are genetic and environmental factors that affect a given person's threshold of toxicity (Vesell, 1991). For example, alcohol abuse and prolonged fasting may be associated with enhanced susceptibility to acetaminophen toxicity. Alcohol acetaminophen syndrome is emerging as an important cause of FHF in the United States. The syndrome is characterized by extremely high transaminase levels and a poor prognosis (Bray *et al.,* 1991). Early treatment with an antidote called N-acetyl cysteine (NAC) is life-saving.

2.1.1.3 Idiosyncratic drug reactions

These are drug reactions which occur rarely and unpredictably amongst the population. Symptoms of idiosyncratic drug reactions are different than the pharmacological effect of the drug. Reactions resulting in FHF include non-steroidal anti-inflammatory drugs (Lewis, 2005) and antitubercular drugs (Smith *et al.,* 1998). Uses of drug combinations which result in FHF are of greater concern than single agents. These include trimethoprim-sulfamethoxazole, rifampicin-isoniazid and isoniazid-acetaminophen.

2.1.1.4 Mushrooms

Amanita phalloides, a species of mushroom also called the "death cap", contains very potent liver toxins that cause severe liver damage (Lionte *et al.,* 2005). Eating Amanita

phalloides results in a syndrome of severe nausea, vomiting, diarrhea and abdominal pain that typically begins about 8-16 hours after eating the mushrooms following which fulminnat liver damage occurs.

2.1.1.5 Other conditions

Several other conditions that affect the liver can cause FHF. In autoimmune hepatitis the immune system of the affected individual attacks the liver (Alvarez *et al.*, 1999). Autoimmune hepatitis usually responds to immunosuppressive therapy, but treatment may not be successful when the patient has advanced liver failure. FHF may rarely occur in pregnancy (Patra *et al.*, 2007), usually during the last trimester. Most patients with pregnancy-related FHF recover following prompt delivery of the infant.

2.1.2 Symptoms

Most patients who develop FHF become ill very rapidly, and within a week or less from the onset of illness near total liver collapse may occur. The early symptoms of liver failure are nonspecific and similar to symptoms of many other conditions. Some of the most common initial symptoms of liver failure are (Yoshiba, 1998):

- Nausea , Loss of appetite, Malaise, Fatigue, Abdominal discomfort, Diarrhea
- Jaundice and scleral icterus (yellowing of the eyes) are often present, but may not be initially noted by patients or their families until relatively late in the course of the illness.
- Encephalopathy, the cognitive dysfunction that is an end result of FHF, is initially subtle. Minor changes in personality, particularly irritability, inattention, mild memory lapses, and insomnia may be the first signs. However, encephalopathy may

dramatically and suddenly worsen, culminating in hepatic coma in a relatively short time.

- Other symptoms of fulminant hepatic failure include: a build up of fluid in the abdomen, which causes swelling called ascites, oedema, a tendency to bruise or bleed easily, fever, itchy skin, dark urine, sleepiness, coma
- As the condition progresses, it causes confusion and erratic behavior as the build up of toxins in the blood affects brain function. Liver failure can also cause kidney failure, coma, and death.

2.1.3 Diagnosis and evaluation

Obtaining a detailed and accurate medical history from patients with FHF is challenging, due to the presence of an altered mental status. Thus, the clinician is usually forced to rely on others to obtain information about recent symptoms, use of medication, risk factors for viral hepatitis (e.g., intravenous drug use, recent tattoos, recent travel, exposure to other ill individuals), and any significant past medical problems. Physical examination is done focusing primarily on determining the stage of encephalopathy, excluding chronic liver disease, and establishing the etiology of FHF (Subramanian *et al.,* 2005). Measurement of prothrombin time and careful evaluation of mental status is carried out in patients with clinical or laboratory evidence of moderate to severe acute hepatitis. If the prothrombin time is prolonged by 4–6 seconds or more and there is any evidence of altered sensory response, the diagnosis of FHF is suspected (O'Grady *et al.,* 1989). Initial laboratory examination must be extensive so that the etiology and severity of liver failure are efficiently evaluated.

Initial laboratory analysis includes the following tests or estimation. Prothrombin time; Complete blood count; Liver function test: AST, ALT, alkaline phosphatase, GGT, Total bilirubin, Albumin; Creatinine, Urea/blood urea nitrogen; Sodium, potassium, chloride,

bicarbonate, calcium, magnesium, phosphate; Glucose; Lipase; Lactate; Blood type and screen, Paracetamol (Acetaminophen) level; Toxicology screen; Viral hepatitis serologies: anti-HAV IgM, HBSAg, anti-HBc IgM, anti-HEV; Autoimmune markers; Immunoglobulin levels; Ceruloplasmin Level (when Wilson's disease suspected); Pregnancy test; Ammonia. Depending on the purpose of the the laboratory tests, they may be classified into 3 groups **(Table 2.1.3.1)**.

Table 2.1.3.1 Classification of laboratory tests for fulminant hepatic failure

Purpose	**Tests**	**Observations**
Determine severity of liver failure	Prothrombin time Bilirubin, Albumin Liver transaminases And other enzymes	The prothrombin time is the single most useful test to determine how the patient recovers from FHF.
Determine cause of FHF	Viral serologies Autoimmune serologies Acetaminophen levels Ceruloplasmin Serum copper Pregnancy test	Determining the etiology quickly and accurately is important as prognosis of recovery depends in part on the cause of FHF. Also some causes FHF may respond to specific therapy.
Predict complications of FHF	Serum Creatinine , Blood urea Hemoglobin, Blood culture White blood count, Urine culture, Glucose Electrolytes, Ammonia	Various lab tests are done to determine whether renal failure, anemia, infection or other problems have complicated FHF. The ammonia level in blood may help in determining the risk of developing cerebral edema.

2.1.3.1 Blood tests and serum liver markers:

A series of blood tests can determine the presence of liver disease. Bilirubin is produced by the liver and excreted in the bile. Elevated levels of serum bilirubin often indicate an

obstruction of bile flow or a defect in the processing of bile by the liver (Yoshiba, 1998). A low level of serum albumin is associated with FHF apart from other liver ailments like chronic liver failure and cirrhosis (Baichoo & Samson, 2006). Elevated levels of serum alkaline phosphatase, the enzyme found in bile usually indicate an obstruction to bile flow and liver injury (Green & Flamm, 2002). Serum aminotransferases, AST and ALT are released into circulation from damaged liver cells (Kew, 2000). The enzyme gamma glutamyl transaminase is liver specific and is released into circulation when the liver is damaged (Karan, 2009). The majority of the coagulation factors are manufactured by the liver and hepatic failure will lead to the depletion of these factors (Blonski, 2007). In addition to impaired hepatic metabolism the absorbance of fat soluble vitamin K through the bile acid system can be impaired creating an effective vitamin K deficiency and inadequate activation of the vitamin K dependent clotting factors (Kowdley *et al.,* 1997). Liver cell damage as seen in FHF interferes with blood clotting that enhances the prothrombin time, the time it takes for blood to clot. Liver health and function should be optimal for production of urea through urea cycle that takes place in liver. Thus urea level in blood gives an indication of liver function and is used to assess prognosis in FHF (Jalan & Lee 2009).

2.1.3.2 Viral serology tests:

Viral serology tests are performed to check if the FHF is caused by viral hepatitis (Gimson *et al.,* 1986). HAV IgM, HEV IgM detect active infection by hepatitis A or E virus. HCV antibodies detect evidence of infection by Hepatitis C virus. HBsAg, HBeAg, and HBV DNA detect infection with hepatitis B virus (Gimson *et al.,* 1983). Anti HBs provides evidence of good immunity to hepatitis B virus infection.

2.1.3.3 Imaging tests

2.1.3.3.1 Abdominal ultrasound: A diagnostic ultrasound is performed to diagnose an injury or disease of the liver, gallbladder, spleen, pancreas, kidneys, or other organs inside the abdomen. In FHF, an abdominal ultrasound is performed to assess the liver, to verify that its vascular supply is intact, and to rule out masses or other problems.

2.1.3.3.2 Computerized tomography scan (CT scan): CT scan is a way to examine internal organs without surgery through which cross-sectional images of structures can be visualized inside the body.

2.1.3.3.3 Liver tissue biopsy: A liver biopsy involves removal of sample of liver tissue for diagnostic purposes. Through a small incision and a hollow-tube needle, the sample is drawn in a quick and painless method.

2.1.4 Pathology

In majority of FHF cases there is widespread hepatocellular necrosis beginning in the centrizonal distribution and progressing towards portal tracts (Boyer and Klatskin 1970). The degree of parenchymal inflammation is variable and is proportional to duration of disease. Most frequently, in FHF, liver shows confluent necrosis, though there is considerable variability in the extent of liver injury. There are no differences in the histopathology corresponding to different etiologies (Hanau *et al.,* 1995). Kuramoto *et al.,* 1991 described a case of FHF following congestive heart failure in which the liver was severely atrophied with massive centrilobular necrosis following which manifestations of hepatic failure like jaundice, encephalopathy, haemorrhagic rashes etc were seen within a week. In FHF due to tumor infiltration, liver histology showed widespread hepatocellular necrosis where the clinical course was of rapid deterioration and death from multiorgan failure (Rowbotham *et al.,* 1998). Histology of liver in FHF developed as an idiosyncratic drug reaction with antiepileptic medication shows hepatic necrosis, with hepatic lobules showing inflammatory

infiltrate with lymphoytes (Albataineh & Siddiqui, 2007). Acute fatty liver of pregnancy is characterized by FHF associated with characteristic pathologic changes of hepatocytes, i.e., microvesicular fatty degeneration with severe mitochondrial swelling (Saibara *et al.,* 1994). The pathogenesis in viral-induced hepatitis -including cell necrosis, inflammation, fibrosis, and cirrhosis formation-is reasonably well understood, and this knowledge assists both in prognosis and in monitoring of therapy (Popper 1975).

2.1.5 Clinical consequence

2.1.5.1 Cerebral oedema and encephalopathy

In FHF, cerebral oedema leads to hepatic encephalopathy, coma and eventually death (Wijdicks*et al.,* 1995). Detection of encephalopathy is central to the diagnosis of FHF. It may vary from subtle deficit in higher brain function (e.g. mood, concentration in grade I) to deep coma (grade IV) (de Carlis *et al.,* 2001). The causative factors remain unclear but are likely to be a consequence of several phenomena (Hazell *et al.,* 1999). There is a build up of toxic substances like ammonia in the brain that affects neurotransmitter level and neuroreceptor activation (Shawcross *et al.,* 2010). Autoregulation of cerebral blood flow is impaired and is associated with anaerobic glycolysis and oxidative stress (Stamelou *et al.,* 2009). Neuronal cell are susceptible to these changes which swell (Larsen and Wendon 2002), resulting in increased intracranial pressure (Koutsilieri *et al.,* 2002). Cerebral edema in FHF results primarily from astrocyte swelling rather than a leaky blood brain barrier (Ranjan *et al.,* 2005).

2.1.5.2 Coagulopathy

Coagulopathy, a disorder in which blood is either too slow or too quick to coagulate, is another cardinal feature of FHF (Gotthardt *et al.,* 2007). Liver has central role in synthesis of coagulation factors. Hepatocellular necrosis leads to impaired synthesis of many coagulation

factors (Kaul and Munoz, 2000). This prolongs prothrombin time which is widely used to monitor severity of hepatic injury. Electron microscopy of platelet**s** from patients with fulminant hepatic failure show structural abnormalities including numerous pseudopods, vacuoles and blurred plasma membranes. In a study by Rubin *et al.,* 1977, it was shown that as patients recovered from fulminant hepatic failure**,** platelet function improved and platelet**s** with normal ultrastructure appeared amongst the abnormal ones.

2.1.5.3 Renal failure

Renal failure is common, present in more than 50% of FHF patients, (Ring-Larsen and Palazzo, 1981) either due to original insult such as paracetamol resulting in acute tubular necrosis (Cobden *et al.,* 1982) or from impaired circulation leading to hepatorenal syndrome (Barada, 2004) or functional renal failure (Fernández *et al.,* 2003). Because of defective production of urea in liver, blood urea does not represent degree of renal impairment but is an indicator liver function.

2.1.5.4 Inflammation and infection

About 60% of all FHF patients fulfil the criteria for systemic inflammatory syndrome (Leithead *et al.,* 2009, Rolando *et al.,* 2000) irrespective of presence or absence of infection. This often contributes towards multi organ failure (Bown *et al.,* 2003). Impaired host defence mechanism due to impaired opsonisation, chemotaxis and intracellular killing substantially increase risk of sepsis (Larcher *et al.,* 1982). Bacterial sepsis (Dirix *et al.,* 1989) mostly due to gram positive organisms and fungal sepsis (Kung *et al.,* 1995) are observed in up to 80% and 30% patients respectively.

2.1.5.5 Metabolic derangements

Hyponatraemia (Bernstein & Tropodi 1998) is a constant feature due to water retention and shift in intracellular sodium transport from inhibition of Na/K ATPase (Papadakis *et al.,* 1990). Hypoglycaemia due to depleted hepatic glycogen store and hyperinsulinaemia (Martin & Pappas, 1990), hypokalaemia, hypophosphataemia and metabolic alkalosis (Calvo & Park, 1996) are often present. Lactic acidosis occurs predominantly in paracetamol overdose (Zabrodski & Schnurr 1984).

2.1.5.6 Haemodynamic and cardio-respiratory compromise

Hyperdynamic circulation with peripheral vasodilatation due to low systemic vascular resistance leads to hypotension (Fernández-Rodriguez 1998). Adrenal insufficiency has been documented in 60% of FHF (Harry *et al.,* 2002) and is likely to contribute in haemodynamic compromise. There is also abnormal oxygen transport and utilization (David *et al.,* 1985). There is a decrease in tissue oxygen uptake, resulting in tissue hypoxia and lactic acidosis (Zabrodski & Schnurr 1984). Pulmonary complications occur in up to 50% patients (Mostafa *et al.,* 2006). Severe lung injury and hypoxemia result in high mortality. Pulmonary haemorrhage, pleural effusions (Trewby, 1978), and intrapulmonary shunts also contribute to respiratory difficulty (Fordham *et al.,* 1998).

2.1.6 Treatment options

Treatment for FHF often involves admission to an intensive care unit and is based upon the cause and the symptoms. Supportive treatment is with adequate nutrition, mechanical ventilation and intracranial pressure monitoring (in severe encephalopathy) (Bernuau & Durand, 2006), and treatment aimed at removing the underlying cause such as acetylcysteine for paracetamol poisoning (Pol & Lebray 2002). The administration of intravenous fluid

restores glucose levels and fluid and electrolyte balance (Russell *et al.*, 1987). Medications, blood transfusions (Ramos & Almario 1990), and hemodialysis (Berger *et al.,* 2000) can be used to remove toxins from the body. While many people who develop FHF recover with supportive treatment (Yoshiba, 1998), liver transplantation (Sass & Shakil 2005).is required in people who continue to deteriorate or have adverse prognostic factors (Bernuau *et al.,* 1986). Various measures to replace normal liver function are evolving as a treatment modality and is gradually being introduced in the care of patients with liver failure. Use of hepatoprotective drugs is increasingly being used to restore liver function (Gong, 2010 (article in press), Kim *et al.,* 2008 and Sinha *et al.,* 2007).

2.1.7 Prognosis

The liver performs myriads of vital functions including processing proteins, sugars, fats, and vitamins; removal of toxic substances (e.g., ammonia); production of bile acids, required for normal digestion; synthesis of clotting factors that prevent bleeding. Consequently, when the liver fails suddenly, the result is a devastating illness. The overall survival for FHF patients in the pre-transplant era was less than 10% (Rakela *et al.,* 1985) and mortality has been very high, being in excess of 80%. In recent years the advent of liver transplantation and multidisciplinary intensive care support has improved survival significantly (Ostapowicz *et al.,* 2002). Though transplantaion has improved mortality, the procedure is ridden with issues, which calls for alternate methods of treating FHF.

2.1.8 Prevention

Since there are multiple causes of FHF that all lead to essentially the same syndrome, no single measure is likely to be effective in preventing all cases. However, several measures can be envisioned that, if successfully executed, should significantly decrease the incidence

of FHF. For example, vaccination for hepatitis A and B has probably contributed to the declining incidence of FHF from viral hepatitis (Mathur & Arora, 2008). Public health initiatives, including guidelines regarding appropriate food handling, (Fitzsimons *et al.,* 2010) also have contributed by reducing the incidence of food-borne hepatitis A.

What does the future hold for FHF?

FHF is potentially reversible (Kobayashi *et al.,* 2009). The FHF patient's outcome depends on the balance between liver injury on the one end and liver regeneration and repair on the other. If the liver injury can be attenuated, or the liver repair and regenerative responses can be enhanced, then recovery is likely. Use of hepatoprotective drugs to counteract the manifestations of FHF is a promising field and a lot of research is currently on in this area. Also recent advances in molecular and cell biology have resulted in the identification of molecular targets that might be manipulated achieve this goal (Mor, 2001).

2.2 Taurine

Taurine, or **2-aminoethanesulfonic acid**, is an organic acid. It is also a major constituent of bile (Bellantini 1987) and can be found in the lower intestine and in small amounts in the tissues of many animals, including humans. Taurine is a derivative of the sulfur-containing (sulfhydryl) amino acid, cysteine. It is one of the few known naturally occurring sulfonic acids. Taurine is named after the Latin *taurus*, which means ox, as it was first isolated from ox bile in 1827 (Tiedemann & Gmelin, 1827) Taurine is not a typical amino acid as it lacks a carboxyl group and in its place contains a sulfonate group and may be called an amino sulfonic acid. Small polypeptides have been identified which contain taurine, but no aminoacyl tRNA synthetase has been identified as specifically recognizing taurine and capable of incorporating it into a tRNA. Taurine plays an important role in the functions of the body (Huxtable, 1992). Absence of taurine does not result in immediate deficiency and disease, but long-term deprivation can cause many health problems.

2.2.2 Structure

The taurine molecule (H2N-CH2-CH2-SO2H) is small and consists of hydrogen (H), nitrogen (N), carbon (C), sulfur (S) and oxygen (O) **(Fig 2.2.2.1)**. Most amino acids have a L- or D-configuration, which means the molecule when put into a solution will rotate light either to the left (Levo=L) or the right (Dextro=D). Taurine, like the amino acid glycine does not polarize light and consequently it does not have an L- or D-configuration. It occurs in the body as a free molecule and is never incorporated into muscle proteins. The taurine molecule

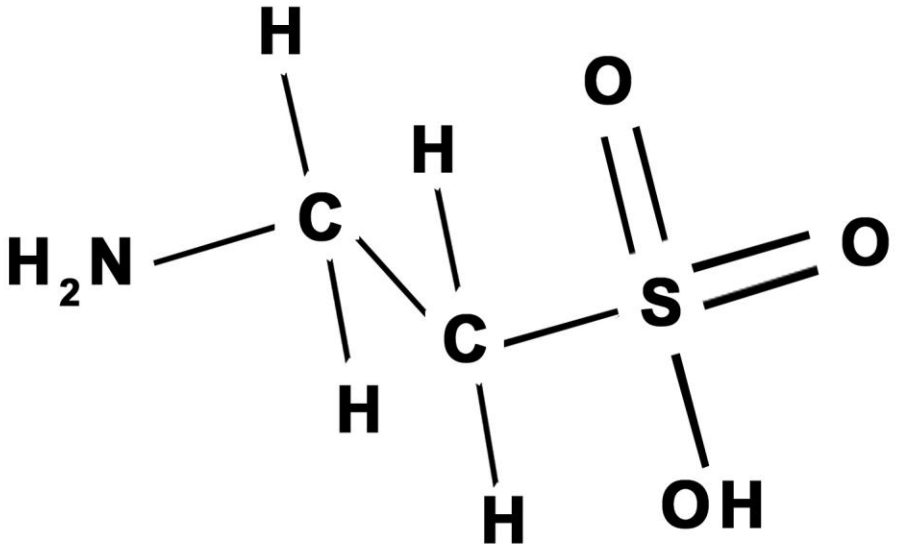
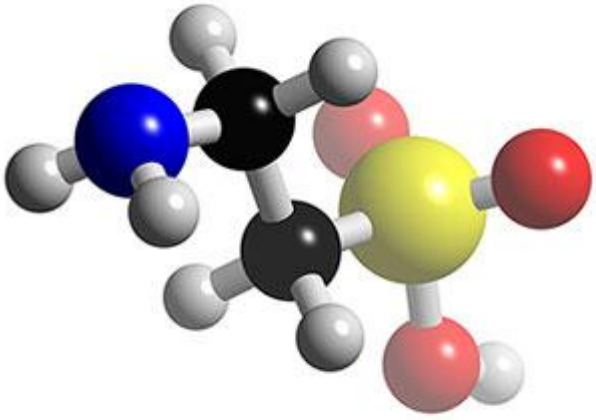


Fig. 2.2.2.1 Ball and stick diagram and chemical structure of taurine mlecule



is water soluble and thus doesn't easily cross the mostly fatty membranes of the body's cell but it is present in all membranes (Löpez-Colomé & Pasantes-Morales 1981).

2.2.3 Occurrence in nature

Taurine is a phylogenetically ancient compound with a disjunct distribution in the biosphere. It is present in high concentration in algae and in animal kingdom, including insects and arthropods (e.g. Allen & Garrett, 1971; Huxtable, 1992; Yin *et al.*, 2000). It is generally absent or present in traces in the bacterial and plant kingdoms. In many animals, including mammals, it is one of the most abundant of the low-molecular-weight organic constituents. A 70-kg human contains up to 70 g of taurine. Taurine is found in greater concentrations in all animal products. Meat, poultry, eggs, dairy products, and fish (Allen & Garret 1971) are good sources of taurine. **Table 2.2.3**.1 shows the level of taurine content present in some seafood. In the animal kingdom taurine is found abundantly in tissues that are excitable, rich in membranes, and that generate oxidants. Thus, it is the most prevalent of all the amino acids in the tissues comprising the skeletal and cardiac muscles and the brain. It is critical to the proper function of the brain, heart, lungs, kidney and blood. Because it performs key functions in cholesterol metabolism related to bile acids, it is essential to the role of the liver, pancreas, and gall bladder.

2.2.4 Properties of taurine

2.2.4.1 Physio-chemical properties

The physio-chemical properties of taurine are given in **Table 2.2.4.1.**

2.2.4.2 Physiological properties

For a long time, taurine was considered a nonessential nutrient for humans. However it is increasingly recognized that taurine plays several important roles in the body being involved in a number of metabolic processes and is essential to newborns of many species. Gaull

Table 2.2.3.1 Taurine content of some aquatic organisms

S.No.	Aquatic organism	mg/100g
1.	Spiral shell	520
2.	Conch	851
3.	Scallop	332
4.	Blood clam	439
5.	Mussel	349
6.	Prawn	143
7.	Crab	279
8.	Cuttle fish	673
9.	Octopus	380
10.	Ray	280
11.	Conger pike	193
12.	Flat fish	314
13.	Journje fish	256
14.	White Chinese Croaker	187
15.	Yellow Croaker	90
16.	Spotted maigre	225
17.	Baby croaker	64
18.	Silver pomfret	41
19.	Hairtail	75
20.	Yellow crucian carp	240
21.	Black snapper	196
22.	Grass carp	185
23.	Silver carp	90
24.	Gucian carp	205
25.	Variegated carp	94
26.	Shell fish	332
27.	Eel	91
28.	Inkfish	672

Courtesy: Zhao Xi-he. Dietary Protein and Amino Acids and their Relation to Health. Asia Pacific J. Clin. Nutr. 1994, 3: 131-134.

Table 2.2.4.1 Physio-chemical properties of taurine

Molecular formula	$C_2H_7NO_3$ S
Molecular weight	125.15
Physical state	Large monoclinic prismatic rod shaped crystals.
Colour	White crystals
Odor	Odorless
Solubility	Soluble in water and insoluble in absolute alcohol.
Melting point	300^0C
pH (0.5M in water, 25^0C)	4.5-6
Optical rotation	Nil

(1989) suggests that since man has low cysteinsulfinic acid decarboxylase, an enzyme necessary for the formation of taurine from cysteine, people are dependent upon dietary taurine. Under certain conditions of high stress or in disease states the need for taurine may increase (Boelens *et al.,* 2003). Several studies reported that plasma taurine concentrations decrease in response to surgical injury (Chirala *et al.*, 1989), trauma (Neary *et al.*, 1997), sepsis, and critical illnesses (Paauw & Davis 1990). As metabolic patterns progressively deteriorate, plasma taurine concentrations decrease severely, suggesting an increased expenditure and possibly an increased requirement for taurine. Taurine along with GABA and glycine is an inhibitory type of amino acid (Saransaari & Oja, 1998), contrary to the excitatory amino acid like aspartate, glutamate and glutamine. Taurine is important in the visual pathways, the brain and nervous system, cardiac function, and it is a conjugator of bile acids. Basically, its function is to facilitate the passage of sodium, potassium, calcium and magnesium ions into and out of cells and to stabilize the charged cell membranes. Another important function of taurine is detoxification. Taurine is required for efficient fat absorption

& solubilization. Studies also showed that dietary taurine supplementation ameliorates experimental renal disease including models of nephrotic syndrome and diabetic nephropathy. The benefinical effects of taurine are mediated by its antioxidant action. (Trachtman *et al.*, 1995). Taurine may also have an important role in renal development. One study with rats showed protective effect of taurine on induced inflammatory bowel disease. With all these discoveries and more on the horizon taurine research is accelerating rapidly.

2.2.5 Synthesis

Mammalian taurine synthesis occurs in the pancreas via the cysteine sulfinic acid pathway **(Fig 2.2.5.1)** from the amino acids methionine and cysteine (Beetsch & Olson, 1998). Vitamin B-6 (pyridoxal-5' phosphate) is a key cofactor in this process. In this pathway, the sulfhydryl group of cysteine is first oxidized to cysteine sulfinic acid by the enzyme cysteine dioxygenase. Cysteine sulfinic acid, in turn, is decarboxylated by sulfinoalanine decarboxylase the rate-limiting step in the taurine synthesis to form hypotaurine. Hypotaurine is then spontaneously or enzymatically oxidized to yield taurine.

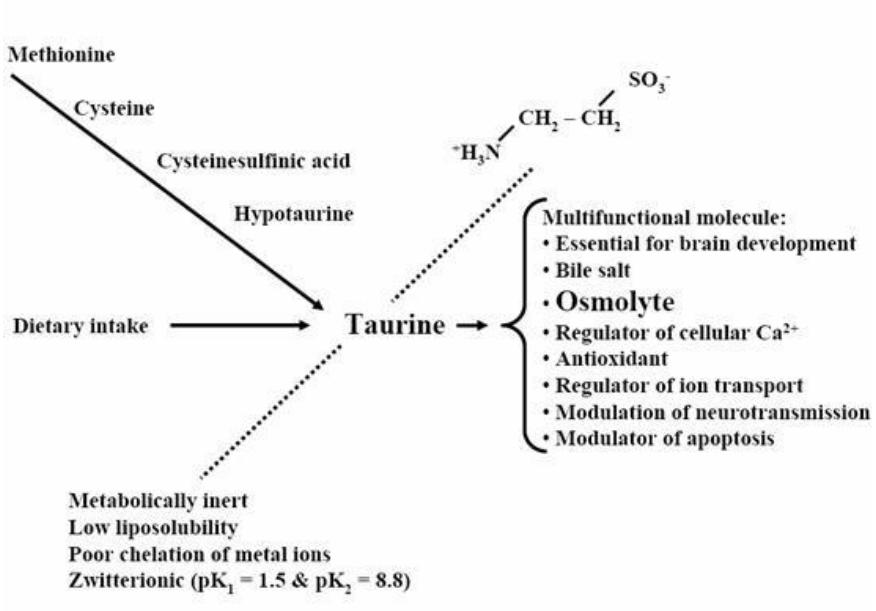


Fig 2.2.5.1 Taurine synthesis and physiological roles in mammalian cells

Courtesy: Regulation of the Cellular Content of the Organic Osmolyte Taurine in Mammalian Cells. I. H. Lambert, Neurochemical Research, Vol. 29, 2004, pp. 27–63

2.2.6 Biological roles

Taurine availability can be affected by the reduction of plasma taurine transport to the cell, restricting dietary supply or using transport inhibitors or antagonists (Braghiroli *et al.*, 1990). Studies of taurine knockout mice have been useful to unravel its role in the development and maintenance of normal organ functions (Warskulat *et al.*, 2007). Such studies have demonstrated significant effects on retinal regeneration (Militante & Lombardini 2004) cardiac dysfunction, cardiac myopathy (Schaffer *et al.*, 2000) and platelet hyperaggregation (Mc Carty, 2004). The decline of tissue taurine content during aging has been demonstrated and could exacerbate age-related increase of oxidative stress and related morbidity (Dawson, 2004). Studies on taurine deprivation have also shown their effect on growth and development. Tauirne is essential for growth and survival of mammalian cells as well as fetus development, development of the newborn and during childhood (Kim *et al.*, 2006).

2.2.6.1 Osmoregulation and cellular tonicity

Cell volume is an essential parameter in the cellular regulation of secretion, metabolism, cell growth and programmed cell death (Zonia & Munnik, 2007). Taurine plays an important role as an organic osmolyte in cell volume control in mammalian cells and a change in the cellular taurine content is an indication of a shift in the cell volume (Lambert, 2004). Cellular volume changes in response to insults like infection, disease, and trauma; restoration of cell volume is essential to recovery from illness (Koshy *et al.*, 1996). Taurine, an osmolyte, helps to regulate osmolarity without causing additional perturbations of cellular tonicity. When cells are hypo-osmotic, they would swell and lyse; taurine is thus extruded to prevent such severe osmolar changes. In hypernatremia, cells are usually shrunken; taurine uptake is thus increased to help regulate osmolarity to prevent possible cell death. This phenomenon was

illustrated by Trachtman *et al.,* 1990, where taurine exerts a protective, osmoregulatory effect on cerebral and extra-cerebral tissues during extreme hyponatremia.

2.2.6.2 Brain health

2.2.6.2.1 Free radicals

Free radicals are highly reactive atoms that wreak havoc in the body by converting stable molecules into unstable ones (Harman, 1992). They can oxidize macromolecules of healthy tissue, causing cell death, mutagenic changes or an increase in unstable substrates such as oxidized LDL, which can readily stick to the lumen of arteries. Free radicals are particularly detrimental to brain tissue, which contains a high concentration of lipids **(**Evans, 1993). Recently, the role of taurine's precursor hypotaurine as a potent antioxidant has been discovered (Fontana *et al.,* 2004; Aruoma, 1998). The sulfinyl group in the hypotaurine molecule is responsible for its efficiency as a radical scavenger. The process by which hypotaurine converts to taurine has been shown to effectively scavenge free radicals and it increases cell viability.

2.2.6.2.2 Hypoxia and ischemia

The central nervous system is least tolerant to hypoxic conditions. Brain death usually occurs in three to five min in an anoxic state. Glucose is one of the basic energy molecules that cells utilize to produce energy, with rapid death of neurons in the hypoglycemic state. A compromise in supplies of both oxygen and glucose results in the condition known as ischemia. Taurine has been shown to prevent the disturbances associated with hypoxia (Michalk, 1997). Taurine modulates the enzymes involved in energy metabolism in the brain, restoring adenine and ATP while reducing ADP and AMP levels.

2.2.6.2.3 Hepatic encephalopathy

Hepatic encephalopathy (HE) is a condition whereby the brain is poisoned by ammonia (Kilburn, 2000). It occurs in association with severe liver damage, where the organ fails to effeciently convert ammonia to urea for excretion (Chatauret & Butterworth, 2004). In addition, concentrations of amino acid precursors to urea that function as excitatory substances such as aspartate and glutamate build up in the body, particularly in the brain (Kojic, 2005). Taurine is redistributed to adjacent cells located in the central nervous system, an attempt to protect those cells from damage (Butterworth, 1996). By its ready availability to CNS cells, taurine's role in cell volume regulation and neuro-protection may be particularly valuable in those suffering from HE.

2.2.6.2.4 Excitotoxicity

Excitotoxicity is the term for the presence of excess amounts of the excitatory amino acids, especially glutamate and aspartate, such that they create an intracellular and extracellular toxic environment resulting in cell death (Chen *et al.*, 1999). To combat this, cells release extra quantities of taurine, known for its volume regulatory ability, thereby buffering the dramatic changes in osmolarity (Wu *et al.*, 2005). Taurine thus acts as both an osmoregulator and neuromodulator (Idrissi & Trenkner, 1999).

2.2.6.2.5 Brain aging

Taurine is found in high concentrations in the brain, though levels decline with age (Idrissi, 2008). Researchers showed that the learning ability of older rats was impaired, impairment correlated to the reduction in taurine levels (Dawson *et al.*, 1999). In cerebrospinal fluid of patients with Parkinson's disease, levels of a few amino acids including taurine are lowered (Engelborghs *et al.*, 2003). Taurine is reported to promote release of

dopamine, a neurotransmitter lacking in Parkinson's disease, from the neuronal pool. Alzheimer's disease, is due to in part, an increase in the generation of nitric oxide (Togo *et al.,* 2004). The supplementation of taurine, along with magnesium inhibits NO production (Chaekyun *et al.,* 2006).

2.2.6.3 Heart health

Taurine makes up nearly 50% of the free amino acids in the heart cells (Huxtable, 1980). Taurine level is depleted in the failing heart (Azuma & Schaffer, 1995). Taurine's electrophysiological actions in cardiac cells are brought about by modulation of ion channels (Satoh, 1998). Calcium homeostasis is critical to stable myocardial contractile function. Changes in the intracellular taurine pool modulate calcium transport and taurine exerts a cardioprotective action (Punna *et al.,* 1994). Through the sodium-calcium transport exchanger in cardiomyocytes, taurine permits the entry of sodium which favors a co-transport of calcium (Harada *et al.,* 1988). It also modulates the activity of calcium channels to promote sodium influx. In these regards, taurine has an essential function in ensuring stable calcium levels, which thereby promotes proper contractile function of the heart tissue. Likewise, potassium is also an important ion in heart cells. Taurine directly modulates the potassium ion current by increasing the current's action potential duration (Satoh, 1998).

In arrhythmia, irregular heartbeat patterns are caused by abnormal extracellular calcium concentration in heart cells. In a research study, the addition of taurine attenuated this response of myocytes to varying calcium concentrations (Takahahsi *et al.,* 1998). Taurine is valuable for its role in protection of heart from oxidative stress and post-ischemic injury (Chapman *et al.,* 1993). It reduces lipo-peroxidation. The ability to scavenge free radicals is a potent cardioprotective role. The quantity of lactate, a marker of ischemic challenge was reduced and quantity of glutathione was enhanced with taurine. ATP levels are also

suppressed in ischemia which was restored by taurine. Through the modulation of lactate, glutathione, and ATP, taurine influences the ability and extent of recovery in heart (Man'kovs'ka et *al* 1998, Timbrell *et al.*, 1995).

2.2.6.4 Effect on skeletal muscle

Taurine is necessary for normal skeletal muscle functioning. This was shown by a study, using mice with a genetic taurine deficiency (Ito *et al.,* 2008). They had a nearly complete depletion of skeletal and cardiac muscle taurine levels. These mice had a reduction of more than 80% of exercise capacity compared to control mice (Geny *et al.,* 2006).

2.2.6.5 Effect on liver health

2.2.6.5.1 Bile

The liver is the site of synthesis of bile which is essential for proper emulsification and thus digestion of fats. Taurine is conjugated via its amino terminal group with chenodeoxycholic acid and cholic acid to form the bile salts sodium taurochenodeoxycholate and sodium taurocholate. The low pKa of taurine's sulfonic acid group ensures that this moiety remains ionized and negatively charged even at the high acidity that occurs at the upper intestine and thus improves the surfactant properties of the cholic acid conjugate (Mende *et al.,* 1999).

In addition to its role in bile salts formation, taurine has considerable importance in cell maintenance functions (Nakashima *et al.,* 1990). As with the neurons and neuroglia, taurine exerts cyto-protective effects when hepatocytes are exposed to hypoxia (Nakashima *et al.,* 1996). According to Nakashima *et al.,* when conjugated with bile acids, taurine increases membrane mobility as well as fluidity. Without proper levels of taurine, the liver cells would be susceptible to osmotic changes and their membranes would become less

permeable (Schaffer *et al.,* 1998). The resulting impairment to the liver would significantly compromise its ability to detoxify blood, allowing toxins to spill into the body.

2.2.6.5.2 Effects on cholesterol

Elevated low density lipoproteins (LDL) are implicated in a range of heart and vascular diseases, including myocardial infarction and artherosclerosis (Holvoet *et al.,* 2003). High density lipoproteins (HDL) are recognized for their protective function on both the heart and vasculature (Karádi *et al.*, 1987). Taurine can attenuate increases in total and LDL cholesterol in people consuming a high fat, high cholesterol diet (Wen *et al.,* 2004) and help reach the favorable lipids ratio. Rats fed a high cholesterol diet plus high doses of taurine demonstrated significant reductions in plasma levels of total cholesterol (32% reduction), LDL cholesterol (37% reduction), and triglycerides (43% reduction) when compared to rats fed a high cholesterol diet without taurine (Park & Lee 1998). Taurine conjugates of all bile acids suppress very low density lipoprotein (VLDL) secretion (Lin *et al.,* 1996). With regard to HDL cholesterol, taurine enhances serum HDL concentration in a dose-dependent manner. In mice, taurine administration lowered serum LDL and VLDL by 44% while elevating HDL by 25% (Murakami *et al.,* 1999). Taurine also decreased the content of cholesterol in the liver by 19%. The cholesterol-lowering action of taurine may lie in its ability to promote the conversion of potentially harmful cholesterol to relatively harmless bile acids. From the studies relating to the ability of taurine to conjugate bile acids and thereby promote fat absorption, a new drug sodium tauroursodeoxycholate to treat cholestasis has been synthesized (Ishizaki *et al.,* 2001).

2.2.6.5.3 Exposures to solvents

Exposure to solvents is common hazard for industrial workers in chemical and petroleum refinement, the plastics and automotive industries, among many others. Solvents have a deleterious effect on the function of the liver and have been linked to birth defects, sterilization, chronic fatigue, etc (Xiao & Levin 2000). Carbon tetrachloride, adversely affects liver function. In a lab study that produced degenerated hepatocytes and necrosis damage from exposure to carbon tetrachloride, researchers discovered that the concurrent administration of taurine could ameliorate the damage (Miyazaki *et al.,* 2005). Taurine moderated the extent and severity of lesions and reduced the number of cancer-antigen positive hepatocytes (Cetiner *et al.,* 2005). Taurine was also shown to protect against DNA (Messina & Dawson 2002) damage which may have prevented the hepatocyte degeneration, lesions, and necrosis characteristic of carbon tetrachloride exposure.

2.2.6.6 Effects on kidney

Taurine is essential for proper kidney function and in its absence, renal capacity is diminished such that the process of excretion of toxic substances from the blood is grossly impaired (Mozaffari, 2003). Taurine acts in the kidney, as an organic osmolyte. Depending on the tonicity of the urine emerging from the kidney, the cells modify their tonicity. When the fluid in medulla is hypertonic, its cells accumulate taurine and similar osmolytes, thus exerting a conservatory effect upon taurine (Fugelli *et al.,* 1995). Trachtman *et al.,* 1995 demonstrated the therapeutic effects of taurine on kidney function of diabetic rats. Taurine administration reduced the total proteinuria and albuminuria by approximately 50%, prevented glomerular hypertrophy, diminished glomerulosclerosis and tubulointerstitial fibrosis, overall ameliorating diabetic nephropathy by reducing renal oxidant injury.

2.2.6.7 Vision

In the healthy eye lens, taurine is found in very high concentration among other amino acids (Heinämäki *et al.,* 1986). Lenses subjected to oxidative stress exhibit characteristic changes in their amino acid profile, with taurine levels greatly depressed and cause changes in lens transparency (Fris *et al.,* 2006). Cataract, the clouding of the clear eye lens, is due to oxidation and glycosylation of proteins in the lens. A lack of the antioxidant nutrients (taurine, vitamins A, C and E and carotenoids) is a major causative factor for the development of cataracts. Taurine acts as an antioxidant by preventing changes in the levels of glutathione, ATP, and insoluble proteins-factors that predispose the formation of cataracts (Devamanoharan 1997).

Taurine plays a critical role in the structure and function of the photoreceptors (Rentería *et al.,* 2004). Through its osmoregulatory function, taurine makes the rod outer segment of the retina resistant to injury. A high-affinity, taurine-specific uptake system is present in the rod outer segment system (Militante & Lombardini, 1999). Through modulation of membrane ion channels, taurine increases calcium uptake to promote the transmission of visual signals from the retina to the brain. Also taurine is important for the regeneration of damaged cells in the retina. It functions to phosphorylate specific proteins and increase cellular outgrowth (Lima & Cubillos 1998).

2.2.6.8 Gasteroenterology

Inflammatory bowel disease is a chronic condition characterized by diarrhea, low-grade fever, fatigue, weight loss, and abdominal cramps (Podolsky, 2002). It is frequently associated with colon ulceration and/or inflammation, which cause an increase in colon weight -- a reflection of tissue edema. Taurine by defending against oxidative damage ameliorates IBD (Mi *et al.,* 1998). Non-steroidal anti-inflammatory drugs (NSAIDs) can

cause gastric ulceration (Wallace, 2000). Taurine by inhibiting neutrophil activation and preventing their adhesion to the gastric lining (Zeybek *et al.,* 2006) and lipid anti-peroxidation effects exerts a protective effect on the intestinal tract.

2.2.6.9 Pulmonary function

The depletion of taurine is harmful to pulmonary tissue. Alveolar macrophages on the surface of lung alveoli become susceptible to oxidative stress when deprived of the antioxidant protective capacity that taurine provides (Minko *et al.,* 2002). In cystic fibrosis (CF), respiratory and digestive systems are affected and are characterized by steatorrhea, indicative of fat malabosrption (Balinsky & Zhu 2004). In children with CF and steatorrhea, taurine supplementation resulted in improved fat digestion, decrease of fecal fatty acid and total sterol excretions and modifiedlipid profile (Carrasco *et al.,* 1990). CF is also marked by liver disease. In a year-long study of CF patients with poor liver function, taurine supplementation caused an increase in serum pre-albumin and restored fat absorption, with no severe side (Colombo *et al.,* 1996). Finally, taurine significantly attenuates endothelial cell apoptosis and necrosis due to oxidative stress (Zhang *et al.,* 2008). These functions are due to antioxidant activity and regulation of intracellular calcium flux. This has great implications for the therapeutic value of taurine in inflammatory-type lung conditions.

2.2.6.10 Inflammatory disorders

In inflammatory disease, plasma taurine becomes depleted, signifying a greater demand by the body in this state (Xu *et al.,* 2008). Taurine prevents the tissue damage that may otherwise result from inflammation. The mechanism involves taurine monochloramine, formed in the leukocytes that inhibit the production of tissue-damaging pro-inflammatory factors like nitric oxide, prostaglandin PGE2, and tumor necrosis factor (Fallahzadeh *et al.,*

2010). The taurine derivative N-chlorotaurine is a weak oxidant produced by leukocytes in response to bacterial and fungal exposures (Neher *et al.*, 2008).

2.2.6.11 Diabetes

In both forms of diabetes - insulin dependent (Type 1) and non-insulin dependent (Type 2), taurine exerts a multitude of beneficial actions. Platelet aggregation in Type 1 diabetes results in an increased risk of cardiovascular incidents. When taurine is supplemented, an increase in both plasma and platelet taurine levels occur that raise the threshold at which aggregation can be triggered (Fianconi *et al.*, 1995). Taurine changes the abnormal blood lipid profile that is associated with the diabetic condition. Researchers found that elevated plasma triglycerides and LDL cholesterol in diabetics were lowered through administration of taurine (You & Chang 1998). In models of diabetic mice, researchers found that taurine supplementation lowered levels of malondialdehyde (MDA), a marker of lipid peroxidation in liver and islets (Lim *et al.*, 1998).

In Type 2 diabetics, the impaired glycemic control is largely due to peripheral insulin resistance, hepatic insulin resistance, and a failure of beta cell function. New complementary therapies including dietary changes, exercise programs, weight loss and supplementation with chromium, vitamin E, magnesium, and soluble fiber can produce improvement in peripheral insulin resistance (Mc Carty, 1997). Recently, taurine has found a role as well, correcting the metabolic anomalies in Type 2 diabetes. Recognizing that these approaches may be adequate but not optimal measures, they are worthy of consideration as adjuncts to drug therapies.

2.2.6.12 Cancer

In cancerous conditions, taurine is a potent cytoprotective agent and immune enhancer (Redmond 1998). Researchers have discovered that taurine inhibits tumors and extends

survival of mice (Schuller-Levis & Park, 2003). Taurine has been shown to be depleted in people taking chemotherapy (Desai *et al.,* 1992). When combined with chemotherapy, taurine extended survival with no tumor visible -- an inhibition rate of 100% (Zhang *et al.,* 1997). Tumor cell membrane fluidity was much improved with taurine supplementation. Recombinant interleukin-2 immunotherapy, in certain types of cancers produces cytotoxic effect on both tumor cells and vascular endothelial cells. When added to the cancer therapy program, taurine acts to reduce endothelial cell death and actually increases the tumor cytotoxicity. The calcium homeostatic mechanism of taurine was found to be the critical feature in these anti-cancer functions (Finnegan *et al.,* 1998).

Hepatocarcinogenesis is marked by changes in lipid peroxidation. Rats exposed to carcinogenic substances without pre-supplementation with taurine, showed depressed glutathione peroxidase activity and membrane stability (You & Chang 1998a). Membrane stabilty and enzyme activity were restored when taurine was supplemented prior to exposure.

2.2.6.13 Prenatal and childhood development

As a key organic osmolyte, membrane stabilizer, and antioxidant, taurine facilitates cellular function right from the first stages of embryonic development. Taurine plays an indirect role in cell division as the process is associated with changes in cell number and cell volume (Fugelli *et al.,* 1995). Deprivation of taurine to embryos proves to be disadvantageous on cellular development, because they rely on inorganic osmolytes for volume regulation and thus are deprived of various cytoprotective functions of taurine. Pregnant women consuming diets that are deficient in taurine place their fetuses at risk for retarded growth (Wu *et al.,* 1998). Taurine is critical during development to produce normal fetal beta cell function (Reusens and Remacle 2006). Taurine has a dose-dependent trophic effect on the human fetal brain cell, promoting proliferation and differentiation. Taurine is indispensable to proper

neurological development and neuromuscular function (Rice, 2000). Taurine is necessary for the proper retinal development in children. Its presence prevents granulation of the retina (Chesney *et al.,* 1998). In the postnatal infant and during subsequent development, taurine continues to dynamically influence the activity of the retina. In neonatal cardiomyocytes, taurine functions as an organic osmolyte (Ying *et al.,* 2009). When taurine is lost, these cardiac cells reduce in size and change in shape as well as configuration to protect against tonicity fluxes.

2.2.6.14 New applications

2.2.6.14.1 Alcoholism

From studies of correlation of taurine with alcohol consumption (Quertemont *et al.*, 1998), the drug acamprosate, calcium salt of N-acetyl-homotaurinate was synthesized. It is specifically designed to maintain abstinence in alcohol-dependent patients and interacts with glutamanergic neurotransmission channels (NMDA receptors) to reduce calcium flux, resulting in a depressed interest in alcohol consumption (Dahchour *et al.,* 1998).

In a study of rats, researchers induced hepatic steatosis and lipid peroxidation by administering alcohol for a period of 28 days (Kerai *et al.,* 1998). However, in the group in which taurine was co-administered, hepatic steatosis was greatly reduced and lipid peroxidation completely prevented.

2.2.6.14. 2 To combat effects of ageing

Decline in taurine levels of the spleen, kidney, eye, cerebellum, and serum are associated with age in rats (Dawson *et al.,* 1999). Taurine supplementation effectively corrects these deficits. Taurine may prove to be important in preserving normal muscle function that is ordinarily compromised with age. In a study of aging rats, depletion of taurine in skeletal

muscle tissue causes decreases in both the electrical and contractile properties. Taurine supplementation significantly raised muscle taurine level, enhancing performance to that of a young rat. Taurine also improves the mechanical threshold for contraction (Pierno *et al.*, 1998). These findings may become applicable for the development of future novel therapies to combat age-related muscular decline.

2.2.6.14.3 Effect on migraine

The underlying biological basis of migraine is yet to be understood. Many of the conditions associated with migraine - neuronal hyperexcitation, vasospasm, hypoxia, platelet activation, and sympathetic hyperactivity (44th Annual Scientific Meeting, American Scientific Society, 2002) - are expected to be countered by increased tissue levels of taurine and magnesium. Some studies state that magnesium taurate may become a valuable drug to reduce migraine incidents (McCarty, 1996).

2.2.6.14.4 Taurine as a marker of disease

In asthmatics, researchers found increased taurine content in bronchoalveolar lavage fluid. Thus, the profile of amino acids in the fluid may serve as a potential diagnostic tool (Hofford *et al.*, 1997). It may be possible to develop specific treatments targeted at modulating the profile of asthmatic bronchial fluid.

2.2.6.14.5 Protection against radiation

Taurine is useful in countering the damaging effects of low levels of gamma radiation. A combination of taurine with vitamin E & C, and alpha lipoic acid has been shown to protect against radiation-associated protein leakage (Bantseev *et al.*, 1997).

2.2.7 Uptake of taurine

The intracellular taurine concentration is a balance **(Fig 2.2.7.1)** between (i) active taurine uptake via the Na^+, Cl^--dependent, pH-sensitive and high affinity taurine transporter TauT, (ii) synthesis from cystein/methionine, and (iii) release via either a transport process that resembles TauT working in reverse or a volume-sensitive taurine leak pathway (Ballatori & Boyer 1992). The total body taurine pool in humans is controlled by TauT located at the brush border of the renal proximal tubule and in the basolateral membrane of the distal nephron (Han *et al.*, 2002).

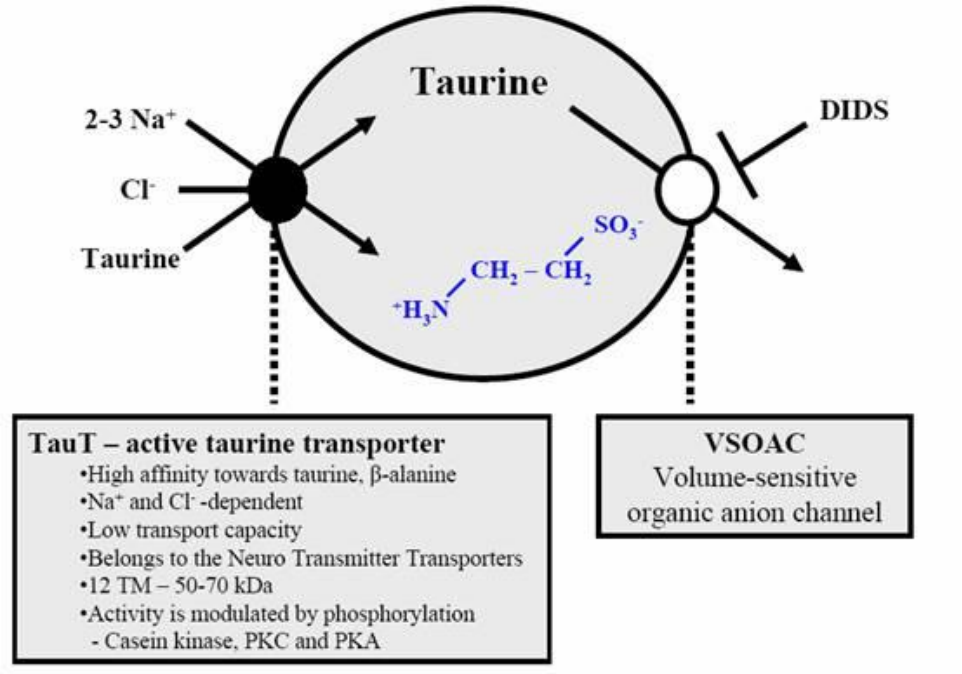


Fig 2.2.7.1 Taurine uptake and release systems in mammalian cell

Source: Lambert IH, *Neurochemical Research* 29: 27-63, 2004

2.2.8 Post absorptive metabolism

Taurine reacts non-enzymically with hypochlorous acid (HOCl) to form N-chlorotaurine (taurine chloramine) and this is then converted to **(Fig 2.2.8.1 & Fig 2.2.8.2)** sulfoacetaldehyde and isethionic acid (8th International Congress on Amino Acids and Proteins, 2003, Cunningham *et al.*, 1998)**.**

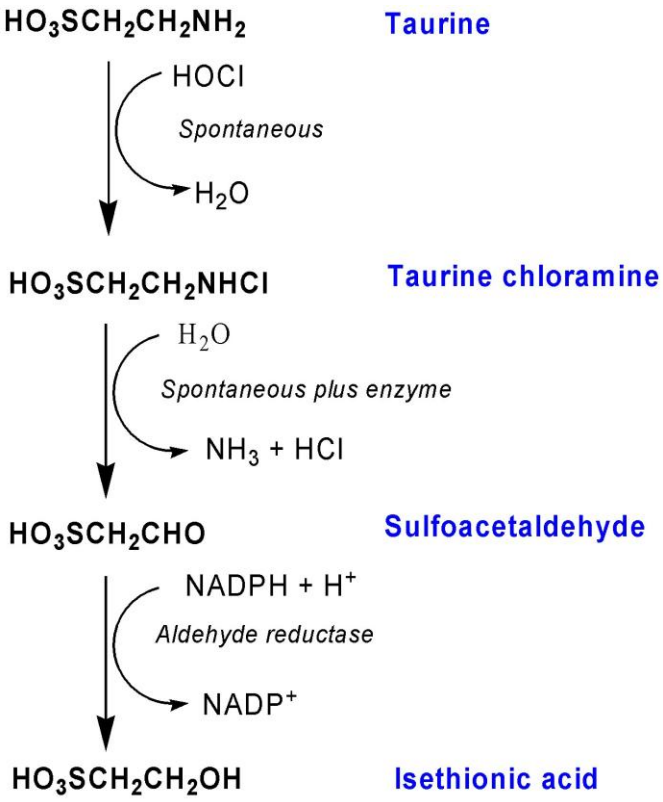


Fig 2.2.8.1 Formation of isothenic acid

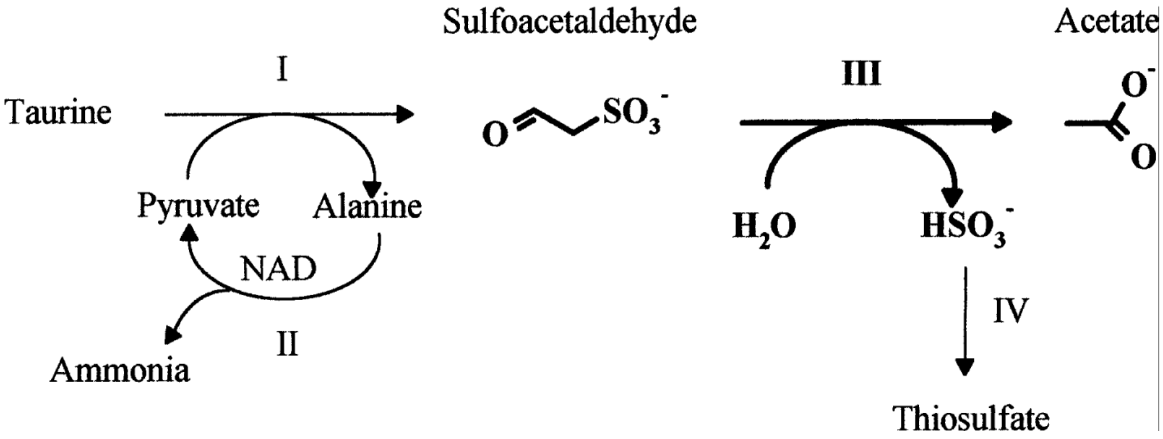


Fig 2.2.8.2 Breakdown of taurine

2.3 D-Galactosamine (D-GalN)

2.3.1 Experimental model

FHF is a severe liver injury accompanied by hepatic encephalopathy which causes multiorgan failure with an extremely high mortality rate, even if intensive care is provided (Fernandez *et al.,* 2003). Management of severe FHF continues to be one of the most challenging problems in clinical medicine. Liver transplantation has been shown to be the most effective therapy. However, the lack of donors combined with the high costs, technical difficulties, viability issues and the disadvantage of needing life-long pharmacological immunosuppressant treatment (Nasseri & Vacanti 2002) following surgical intervention with the added complication that the immunosuppressant agents used themselves produce side effects in the kidneys, liver and other organs (Khan, 2009), mean that liver transplantation is not always an option. For these reasons, other therapeutic options to help patients recover or stabilize have to be considered. Although a number of clinical trials testing different liver assist devices are under way (Tilles *et al.,* 2002, Patzer II 2001, Detry *et al.,* 1999, Chen *et*

al., 1997), these systems alone have no significant effect on patient survival and are only regarded as a useful approach to bridge patients with FHF to liver transplantation. As a result, reproducible experimental animal models (Newsome *et al.,* 2000) resembling the clinical conditions are very relevant. The three main approaches (van de Kerkhove *et al.,* 2004) used to create an animal model for FHF **(Table 2.3.1.1)** are: surgical procedures (total/partial hepatectomy, complete/transient devascularization), toxic liver injury by the use of hepatotoxic drugs (acetaminophen, D-GalN, thioacetamide, and others) and infective (viral models) procedures.

Table 2.3.1.1 Main FHF animal models in different species

Animal model	**Species**	**Advantages/disadvantages**
Surgical		
Total/partial hepatectomy	Pig, dog, rabbit, rat, mouse	Hepatic encephalopathy (HE); reproducible/no reversibility; no long-term survival
Total/partial devascularization	Pig, dog, rabbit, rat	HE; reproducible/no reversibility; no long-term survival
Chemical		
Acetaminophen	Pig, dog, rabbit, rat, mouse	HE; no hazard/non-reproducible; variable interval between damage and death; species and age variability
Amanitin	Pig	HE; specific toxic effects; large animal
Azoxymethane	Mouse	HE; reproducible/small size; hazard
Carbon tetrachloride	Pig, rabbit, rat, mouse	HE /non reproducible; extrahepatic toxicity; small time window before death
Concanavalin A	Rat, mouse	HE /small size
D-Galactosamine	Pig, dog, rabbit, rat, mouse	biochemical markers/non-reproducible; hazard; variable interval between damage and death; species differences, HE
Lipopolysaccharide	Rat, mouse	HE /non-reproducible; small size; hazard; small time window before death
Thioacetamide	Rabbit, rat, mouse	HE; reproducible; large time window before death/hazard
Viral		
Hemorrhagic disease	Rabbit	HE; reproducible; no hazard

Source: Newsome et al. LiverTransplantation,Vol 6, No 1 (January), 2000: pp 21-31

The use of chemical agents such as acetaminophen, thioacetamide or D-GalN (Blei *et al.*, 1992) may reproduce a number of important FHF clinical characteristics, such as hypoglycemia, encephalopathy, and increased blood levels of hepatic enzymes and hepatotoxic chemical agents are frequently used as a model for FHF. Criteria for an FHF animal model are shown in **Table 2.3.1.2**

Table 2.3.1.2 Criteria for an FHF animal model Terblanche and Hickman (1991)	
Reversibility	Suitable treatment may reverse and improve survival
Reproducibility	Reproducible end-points are required to standardize the model
Death from liver failure	Should reflect biochemical, histological and clinical changes including death from AHF
Therapeutic window	Time for treatment should be available between insult and death
Adequate animal size	Size large enough to allow blood and tissue analysis to take place serially
hazard to personnel	Minimum risk for operators and associated staff

D-GalN **i**s a molecule which, when metabolized *via* the galactose pathway in the liver, causes serious metabolic alterations and hepatic necrosis through depletion of different uridine intracellular mediators (Kim *et al.*, 2004), and has therefore been used to develop FHF models. This model also displays the characteristic effects of human FHF (Hung *et al.*, 2007), such as an increase in blood levels of liver enzymes, bilirubin, ammonium or lactate and the associated coagulopathy, hypoglycemia, coma and increase in intracranial pressure. A reproducible model has been developed with pigs (de Groot 1987) which, because of their size, are suitable for the assessment of different support systems designed for treating FHF in humans. Significant differences in D-GalN sensitivity across different species exist (Galanos *et al.*, 1979). Furthermore, the interval between damage caused and death is not uniform, the agent is expensive to use in large-scale models, and lastly, it carries health risks. D-GalN

models have been used to investigate the renal damage which accompanies FHF and the liver metabolic pathways involved. D-GalN models have also allowed testing of different extracorporeal hepatic support devices and bioartificial systems (Frühauf *et al.*, 2004), including hepatocytes transfected with the human gene interleukin-1 receptor antagonist in rats, the use of a nonwoven fabric bioreactor containing porcine hepatocytes, or the study of the potential effects of cerebrospinal fluid drainage and cranial decompression in rats

2.3.2 Structure and properties of D-galactosamine

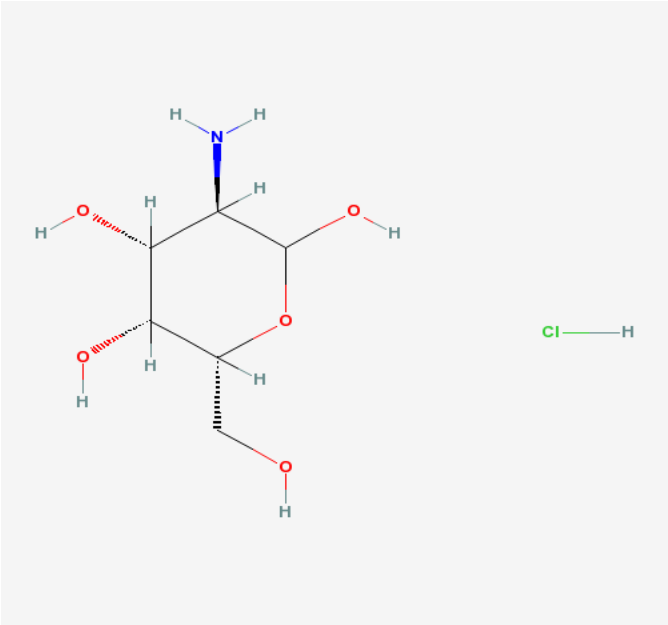


Fig 2.3.2.1 Structure of galactosamine

Chemically D-GalN is 2-amino-2-deoxy-hydrochloride with a molecular formula of $C_6H_{14}ClNO_5$.HCl and molecular weight is 215.63206 g/mol. The hydrochloride salt of D-GalN is a stable white powder soluble in cold water with a melting point 178-180^{0}C. In presence of conditions like excess heat and incompatible materials, the compound is explosive and it is reactive with oxidizing agents (**Fig-2.3.2.1).**

2.3.3 Pathophysiological mechanism of D-galactosamine

D-GalN has been shown to produce a liver damage closely related to human viral hepatitis both biochemically and histologically (Shi, 2008). The mechanism of the hepatotoxicity of D-GalN is as yet unknown, although certain biochemical defects have been reported. These include depletion of liver glycogen and adenine nucleotides (Keppler & Decker, 1969), and depletion of uridine phosphates with accumulation of UDP-hexosamines (Keppler *et al.,* 1970), as well as a decrease in protein synthesis. D-Galactosamine-1-phosphate and UDP-galactosamine were identified as the predominant early metabolites of D-GalN in rat liver (Beckwith-Hall *et al.,* 1998).

Galactosamine-1-phosphate accumulation is enhanced by the strongly reduced levels of UDP glucose. The influence of the strongly diminished UDP glucose levels on the UDP glucose-linked syntheses of glycogen, heteropolysaccharides and glucuronides as well as the trapping of uridine phosphates by formation of UDP-hexosamines may play an important role in the induction of galactosamine hepatitis. Within thirty min of D-GalN administration in rats, there occurs a high accumulation of UDP-galactosamine derivatives in the liver, leading to a depletion of hepatic UTP (Galanos *et al.,* 1979). As a result biosynthesis of macromolecules (RNA, proteins, glycoproteins, glycogen etc) ceases. These alterations lead to eventual cell damage and cell death which at later stages of the reaction may be identified by the increase of liver enzymes in the blood and by histology. The D-GalN-induced liver injury is intimately connected with the alterations in the structure and function of the plasma membrane due to impaired glycoprotein synthesis (Sinha *et al.*, 2007).

2.3.4 Metabolic alterations

Administration of the amino sugar D-GalN to rats causes liver damage, which morphologically resembles acute hepatitis (Lehmann *et al.,* 1987, Keppler *et al.,*., 1970). D-GalN treatment of rats results in a marked increase of liver specific enzyme activity in the

blood of animals (Sugiyama *et al.,* 1999). These enzymes include aspartate aminotransferase, alanine aminotransferase, alakaline aminotransferase, lactate dehydrogenase, gamma glutamyl transferase and glutamate dehydrogenase. Bilirubin concentration, predominantly unconjugated type is elevated making the plasma of galactosamine-treated rats (Lo *et al.,* 1987). Gluconeogenesis from lactate is decreased (Yokoyama *et al.*, 2005) which may be due to the lower activity of the key enzyme, phosphoenolpyruvate carboxylase (EC 4.1.1.32) resulting in hypoglyceamia. Effect of D-GalN on the concentrations of metabolites in blood includes a significant elevation of lactate, pyruvate and 2-oxoglutarate all of which indicate impaired gluconeogenesis (Banta *et al.*, 2005). The activity of several hepatic enzymes is lowered which may be a result of membrane damage (Sugiyama *et al.,* 1998), reduced protein synthesis (Geng *et al.,* 2005), increased rates of intracellular degradation of the enzymes or conversion into inactive forms.

2.3.5 Morphological and structural changes

It is known that administration of D-GalN in large quantities to the rat induces liver injury which shows morphological and functional features similar to those of acute human viral hepatitis. D-GalN produces foci of hepatocellular necrosis scattered throughout the lobule (Sielaff *et al.,* 1995) and accompanied by an inflammatory infiltrate of polymorphonuclear leukocytes and lymphocytes. The picture of progressive inflammatory response with severe liver cell degeneration and necrosis as well as lobular distortion and increased fibrous tissue closely resembled those in human active hepatitis (Tsuji & Shinohara 1981). D-GalN produces acute hepatocellular lesions in rats (Taniguchi *et al.,* 2002) and other animals (Braun *et al.,* 2000, Nayyar & Koenig 1974). Light microscopically, cirrhotic changes were observed (Dhanabal *et al.,* 2006) in most of the animals characterized by the proliferation of the connective tissues from portal triads into hepatic lobules. Features like edema of

hepatocellular microvilli and widening of sinusoidal endothelial fenestrae (SEF); massive hepatic necrosis with hemorrhage are commonly seen. A single intraperitoneal injection of 800 mg/kg or 1500 mg/kg D-GalN-induced remarkable histological and cytological changes in the rat liver (Sinha *et al.,* 2007). Light microscopically, the liver showed diffuse parenchymal damage, (Wan *et al.*, 2008) in which hepatic cell cords were disorganized and a marked accumulation of lipid droplets (Sasaki *et al.,* 1996) were found in the hepatocytes.

2.3.6 Ultrastructural changes

Electron microscopical studies of hepatocytes of mice treated with D-GalN showed-dilation of ER of both rough and smooth type with swollen mitochondria (Datta & Bhattacharya, 2001; Shigeta, 1997). Nuclear changes showed increase in size and striking anisonucleosis, condensation of chromatin, fragmented and dispersed nucleoli in D-GalN-induced hepatotoxic mice (Trump *et al.,* 1973, Boyer & Klatskin 1970).

In an ultra structural study (Takenaka *et al.,* 2007) of D-GalN induced hepatic damage, edema of the hepatocellular microvilli, widening of the smooth endoplasmic reticulam and transmigration of red blood cells (RBC) and platelets to the space of Disse without exfoliation and necrosis of the sinusoidal endothelial cells were observed. Transmigration of RBCs and platelets to the space of Disse resulted in massive hepatic necrosis due to occlusion of the microcirculation (Yi *et al.,* 2006). In a study, (Arai, 2001) transient injury to the rat liver was induced by a single intraperitoneal injection of D-GalN. After the administration of the drug, most of the liver cells showed synchronous morphological alteration of the plasma membrane accompanied by changes in the histochemical localization of several enzymes in the liver cell. In a study involving administration of a single dose of D-GalN (Kouta *et al.,* 1985) liver ultrastructure showed disruption of lamellar arrangement of rough endoplasmic reticulum, dissociation of intrahepatic cell space and an increase in the number of autophagic

vacuoles. Histo- and cytochemical detection of 5'-nucleotidase and alkaline phosphatase activities revealed disruption of the bile canaliculi system and a disturbance of plasma membrane.

An electron microscopic study (Funatsu *et al.*, 1978) of mitochondria demonstrated mitochondrial proliferation and irregularities with crenated membranes, focal hypertrophy of the smooth endoplasmic reticulum, and decrease of the rough endoplasmic reticulum with partial detouchment of ribosomes, loss of compactness of nucleoli and accumulation of lipid droplets in the cytoplasm. The proliferation of collagen fibers was observed around the hepatocytes and acid mucopolysaccharides were seen in the space of Disse and partly in the sinusoids histochemically using electron microscope. D-GalN, injected into the lumbar theca in a dose of 1 mg or 5 mg (Nayyar & Koenig, 1974), produced a lumbosacral myelopathy with hindlimb weakness or paralysis and loss of sphincter and sensory function. Important early changes visible in the light microscope were a perineuronal and perivascular edema and a condensation of glial chromatin. On electron microscopic examination glia were more severely damaged than neurons. The early glial lesions included: (1) distension of the cisternae of the rough endoplasmic reticulum, including the perinuclear cisternae; (2) mitochondrial swelling; (3) astroglial edema with partial uplifting of nerve endings at synapses; (4) nucleolar changes, including loss of granular constituents and fragmentation; and (5) nuclear changes, notably, a clumping of chromatin, and aggregation of nucleoplasmic granules.

2.4 Liver

The liver is the largest organ in the body, weighing 1.2 to 1.8 kg in the adult (Mitra & Metcalf 2009). The gallbladder is attached to the inferior surface. The liver is divided into the right lobe (larger) and left lobes. The connective tissue septa between the lobules hold branches of the hepatic artery and the portal vein, as well as bile ducts. The liver is composed primarily of hepatocytes which are arranged in cords that extend from the central vein to the portal triads. Each hepatocyte is a polygonal cell with a large, centrally located nucleus. In healthy liver cells, deposits of glycogen are seen (Leander *et al.*, 2000). Adjacent liver cells form tight junctions. Each liver lobule is surrounded by a number of portal triads, each consisting of a single branch of the portal vein, a branch of the hepatic artery, and a bile duct. The liver cell cords are separated from each other by sinusoids which contain blood. Numerous Kupfer cells, which are macrophages by lineage, are also present along the sinusoidal space. The liver cells themselves are separated from the sinusoid by a narrow space called the space of Disse (Selden *et al.*, 1999).

2.4.1 Liver cell injury

In case of liver injury hepatocytes swell via increased water content (Farrell *et al.*, 2008). This is termed hydropic change and is a result of membrane damage and impaired mitochondrial function. Fat mainly the neutral fat of triglycerides accumulates, a phenomenon referred to as steatosis. It indicates some defect in lipid metabolism or lipoprotein synthesis or unusual quantities of adipose or dietary lipid brought to liver. There is loss of cellular fine structure or microvilliFrom the nucleus point of view (Dancygier & Schirmacher, 2010), karyolysis (DNA degradation), pyknosis (nuclear shrinkage), karyorrhexis (nuclear fragmentation) occur (Hooser, 2000). Cell injury can be reversible or irreversible **(Fig 2.4.1.1)**.

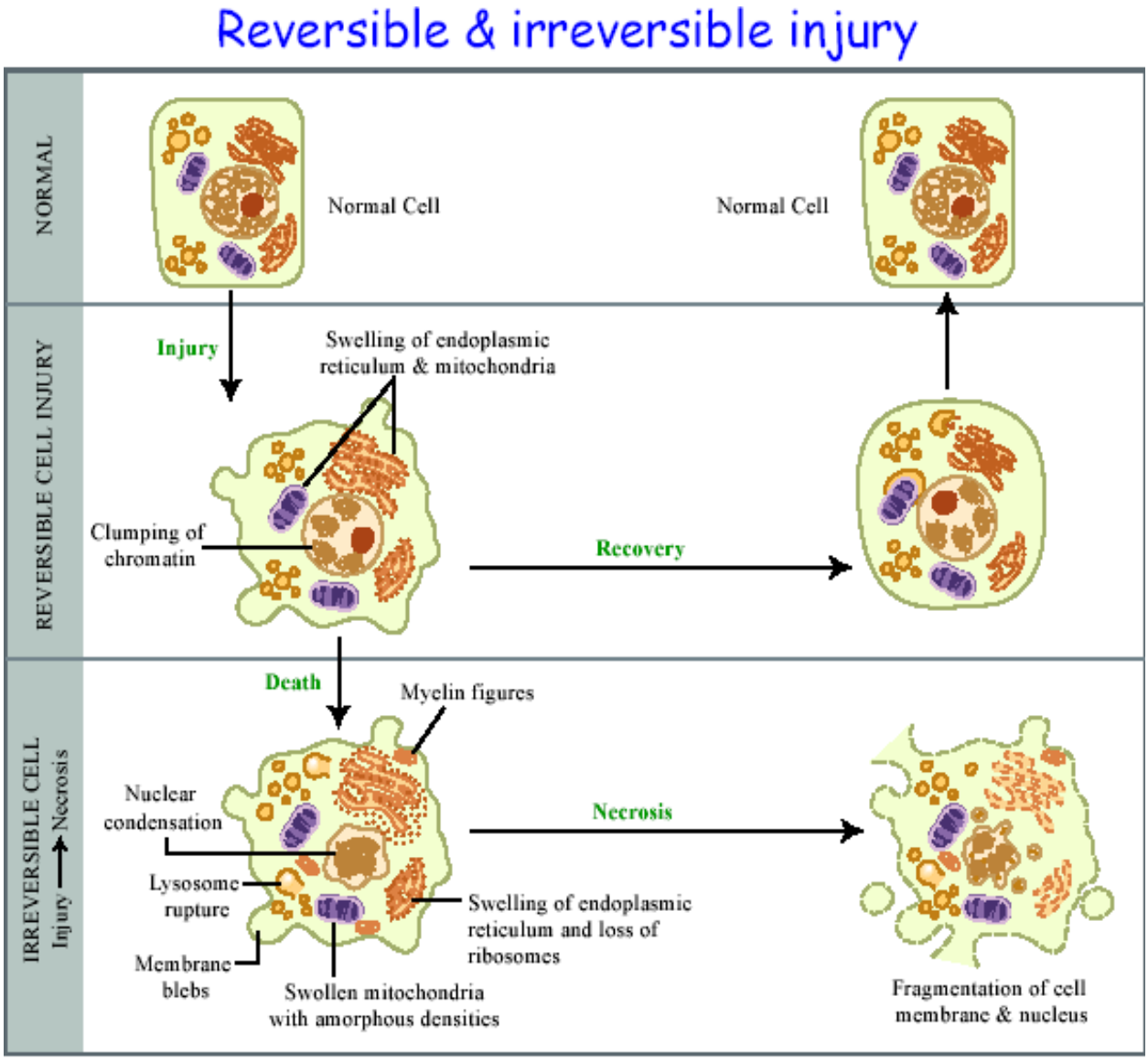


Fig 2.4.1.1 Features of reversible and irreversible injury

Reversible injury is characterized by generalized swelling of the cell and its organelles, blebbing of the plasma membrane (Myagkaya *et al.,* 1984) detachment of ribosomes from the endoplasmic reticulum and clumping of nuclear chromatin (Trump *et al.,* 1996). Transition to irreversible injury is characterized by enhanced swelling of the cell, swelling and disruption of lysosomes, presence of large amorphous densities in swollen mitochondria, disruption of cellular membranes and profound nuclear changes (Trump *et al.,* 2001). The latter include

nuclear condensation, fragmentation and dissolution of the nucleus. Irreversibly injured cell becomes a dead cell either by apoptosis or by necrosis **(Table 2.4.1.1 & Fig 2.4.1.1)**.

Table 2.4.1.1: Features of Necrosis and Apoptosis

Feature	**Necrosis**	**Apoptosis**
Cell size	Enlarged	Reduced
Nucleus	Pyknosis/ karyorrhexis/ karyolysis	Fragmentation
Plasma membrane	Disrupted	Intact
Cellular contents	Enzymatic digestion	Intact
Inflammation	Frequent	None
Physiologic/pathologic	Pathologic	Physiologic

2.4.2 Causes of liver injury or hepatitis

Hepatitis is an inflammation of the liver characterized by diffuse or patchy necrosis (Danet *et al.,* 2003, Semelka *et al.,* 2001). Major causes are specific hepatitis viruses, alcohol, and drugs (Ungo *et al.,* 1998). Less common causes include other viral infections (eg, infectious mononucleosis, yellow fever, cytomegalovirus infection (Stern, 1972) and leptospirosis (Datta & Christopher 2005). Various systemic infections and other illnesses may produce small focal areas of hepatic inflammation or necrosis.

2.4.2.1 Viruses

Acute viral hepatitis is diffuse liver inflammation caused by specific hepatotropic viruses that have diverse modes of transmission and epidemiologies and each type shares clinical, biochemical, and morphologic features. Acute infection tends to develop in predictable phases. This topic has been described in **Introduction**

2.4.2.2 Drug-induced hepatotoxicity

This is a chemical-driven liver damage. Hepatotoxicity can be broadly considered to occur in two forms, type A *symptomatic* or type B *idiosyncratic*. Drugs that have a *symptomatic* hepatotoxicity (Thiim & Friedman, 2003) are those that are common, related to the pharmacological action of the drug and have predictable dose-response curves and well characterized mechanisms of toxicity. In contrast, the second type, *idiosyncratic* hepatotoxins (Deng *et al.,* 2009) are uncommon and cause liver damage in only a small fraction of the population that is exposed to the agent, does not have a clear dose-response.

Most of the drugs are lipophillic substances and they cannot be excreted in bile or urine. They are transformed into hydrophilic metabolites (that may be stable or unstable) by cytochromes, oxygenases present in liver. The unstable and reactive metabolites covalently bind to hepatic macromolecules and cause type A toxicity (Park *et al.,* 2001) **(Fig 2.4.2.2.1)**.

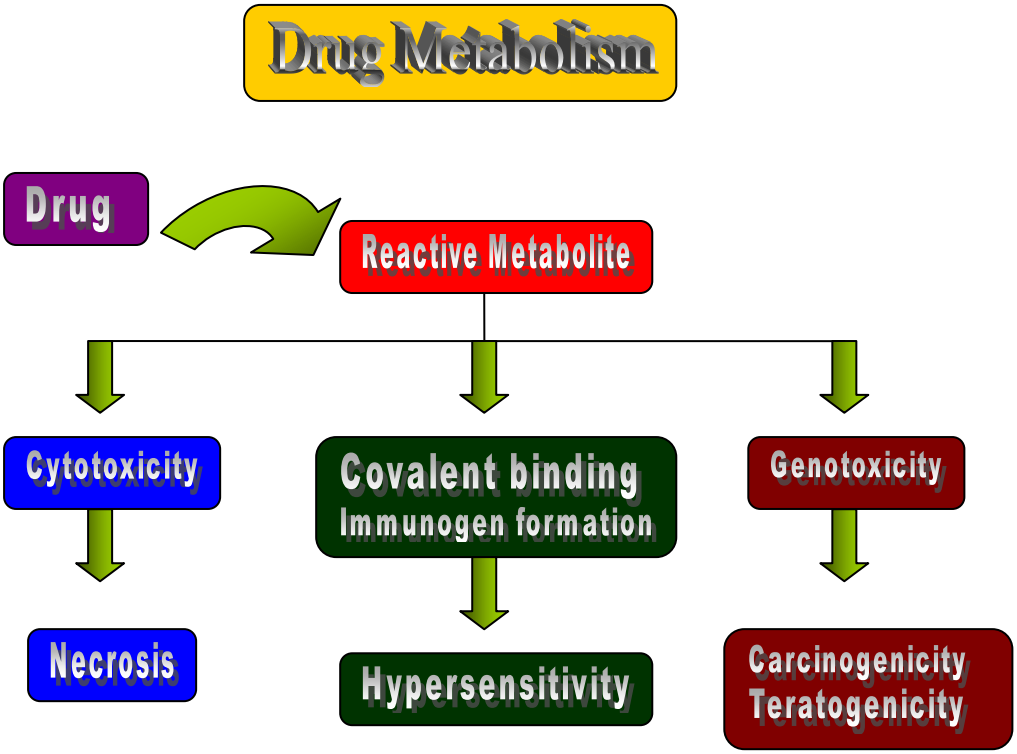


Fig 2.4.2.2.1 Drug-induced hepatotoxicity

The activity of cytochromes is widely influenced by environmental factors such as alcohol, genetics and other drugs, giving rise to potential drug interactions (Zanger *et al.*, 2000). In the hepatocytes, there are various protective mechanisms explaining the fact that only a huge dose of a xenobiotic or a high metabolic activation rate (Slikker *et al.*, 2004) will cause direct type A toxicity.

In addition, the reactive metabolites covalently bind to hepatic proteins resulting in the formation of a protein adduct or an "alkylated protein" which forms a complex with the major histocompatibility complex (Robin *et al.*, 1997). This neo-antigen expressed on the liver cell membrane may then be recognized by an immunocompetent T cell which will be responsible for cytotoxicity and cell necrosis. This phenomenon is likely involved in type B indirect type of hepatotoxicity as seen in acetaminophen toxicity (Mumoli *et al.*, 2006). Some reactive metabolites can also bind to hepatic proteins giving rise to a hypersensitivity reaction (Naisbitt 2001). Finally, some reactive metabolites can induce genotoxicity, giving rise to carcinogenicity and teratogenicity (Luch 2005).

Among the different targets for drug toxicity in the liver, the first target is the hepatocyte and drug-related hepatitis can be acute or chronic **(Fig 2.4.2.2.2)** (Liu, 2009). Some drugs induce prolonged inflammation and even cirrhosis of the liver. In the hepatocyte, mitochondria can be involved in some toxicities and this gives rise to microvesicular steatosis (Pessayre *et al.*, 2007, Fromenty & Pessayre, 1997). Some drugs cause inflammation of the endothelial cells of blood vessels of the liver giving rise to a condition called veno-occlusive disease (Schouten *et al.*, 2008). Finally, some substances can induce an activation of the stellate cells. These cells will be transformed into myofibroblasts giving rise to an extensive fibrosis of the liver (Paik *et al.*, 2009). Many drugs, including isoniazid, methyldopa, nitrofurantoin and, rarely, acetaminophen, can cause chronic hepatitis. The mechanism varies

with the drug and may involve altered immune responses, cytotoxic intermediate metabolites, or genetically determined metabolic defects (Park *et al.*, 2000).

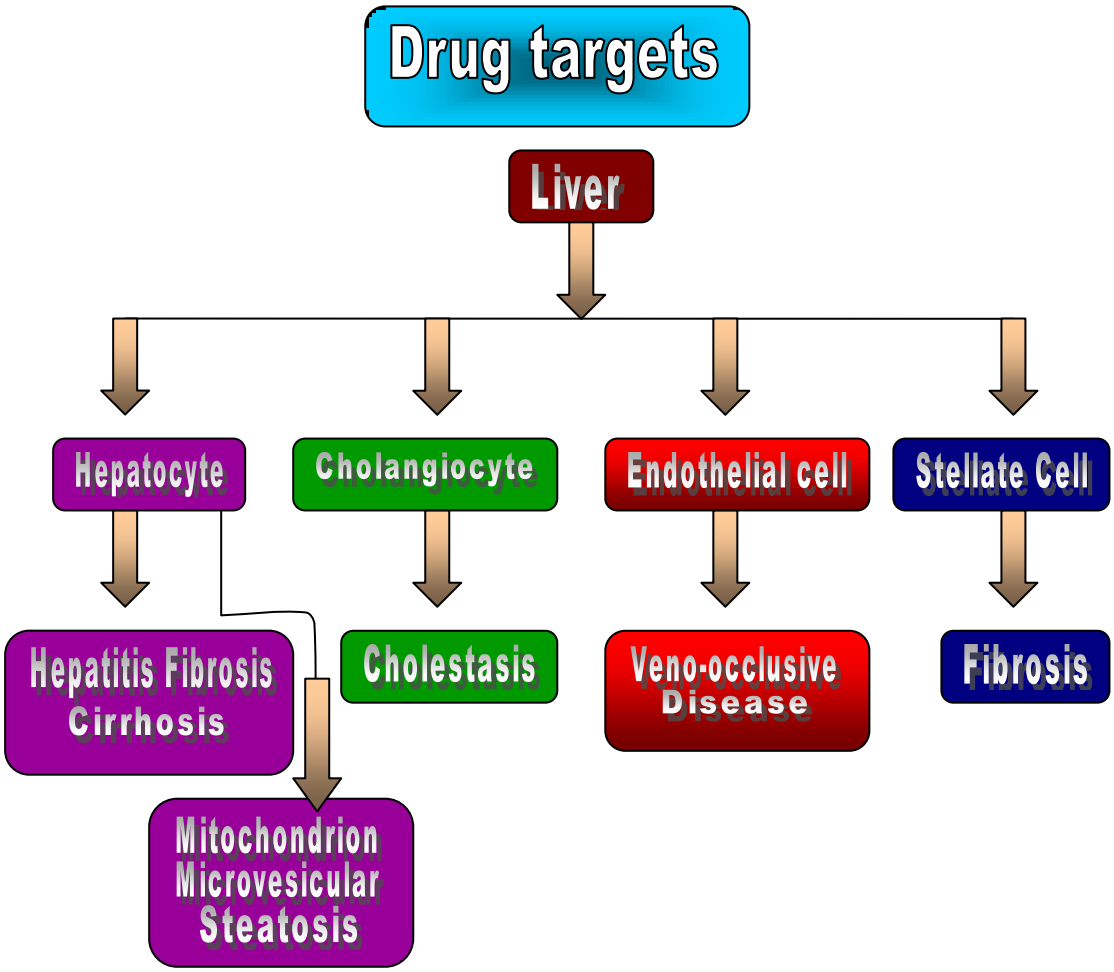


Fig 2.4.2.2.2 Targets for drug toxicity

2.4.2.3 Alcohol-induced liver injury

Alcoholic liver injury is a form of toxicity, with an immune component to some of the injury (Hines & Wheeler, 2004). Pathophysiology involves fat accumulation (steatosis), inflammation, and fibrosis (Brunt 2007). It has a number of very specific, reproducible and easily recognizable features.

2.4.2.3.1 The Fatty Liver of the Alcoholic Chronic consumption of alcohol causes the liver cells to accumulate triglycerides (steatosis) resulting in enlarged livers with rounded edges (Enomoto, 2000). Alcohol and its metabolites affect virtually every aspect of lipid metabolism in the liver. Possible mechanisms for steatosis include reduced synthesis of very low density lipoprotein (VLDL) and increased hepatic triglyceride synthesis (Mensenkamp, 2001). Alcoholic livers tend to accumulate protein as well, presumably as a result of a defect in protein secretion by the hepatocytes (Kharbanda *et al.,* 2007).

2.4.2.3.2 Ultrastructural Changes Chronic alcohol intake causes mitochondrial enlargement and distortion of cristae (Lewis, 2000). Alcoholic mitochondria have defects in Kreb's cycle enzymes and are sluggish energy producers (Hoek *et al.,* 2002). The mitochondria may reach sizes, larger than the nucleus. Expansion of the smooth endoplasmic reticulum, (Gariot *et al.,* 1987), results in enlargement of the liver cells.

2.4.2.3.3 Alcoholic Hepatitis A few alcoholics develop a central zone hepatitis characterized by cellular swelling, spotty necrosis (Sherlock, 1990). Alcoholic hepatitis progresses to more severe forms of chronic alcoholic injury, including cirrhosis (Teli *et al.,* 1995)

2.4.2.3.4 Central Sclerosis Some alcoholics deposit collagen in the spaces of Disse along the central zone sinusoids and around the central vein (Taguchi and Asano, 1998), eventually obliterating that vein. This type of injury is a precursor of alcoholic cirrhosis.

2.4.3 Types of liver injury

Liver diseases are defined by the types and patterns of injury, inflammation, and cholestasis they induce. The basic patterns of injury, as presented below, are those which are continually used by tissue pathologists to make diagnoses of liver disease.

2.4.3.1 *Hepatic necrosis*

Hepatic necrosis is a severe and rapidly progressing form of hepatitis accompanied by hepatocellular death and the signs and symptoms of hepatic failure (Goodman, 2002). Massive and submassive hepatic necroses are the histopathological manifestations of fulminant hepatic failure (Kirsch *et al.,* 2009). Causes are diverse and may be due to infections, intoxications, severe hepatic ischemia, hepatic metabolic disease like Wilson's disease, acute auto-immune hepatitis and others. Grossly, the liver with large areas of necrosis may appear shrunken and yellowish. Typically necrotic cells go through a phase of hydropic degeneration whereby the cell enlarges and its outline becomes irregular, with resultant swelling of the organ followed by rupture of the cell (Levine & Saltzman, 2004). Focal necrosis is followed by an inflammatory infiltrate which results in removal of the necrotic debris and restructuring of the lobular structure, by division of existing cells within the connective tissue framework (Gerlach *et al.,* 2008). Repeated bouts of necrosis and repair may result in disruption of the structure of the liver and result in cirrhosis. Massive necrosis, on the other hand, can result in liver failure and death (Ekici *et al.,* 2005).

2.4.3.2 *Hepatic fibrosis*

Hepatic fibrosis is an accumulation in the liver of connective tissue in response to hepatocellular damage of nearly any cause (Youseff & Tavill, 2002). It results from excessive production or deficient degradation of the extracellular matrix. Hepatic fibrosis can regress if the insult is reversible (eg, viral clearance). More commonly, however, injury is chronic or repeated, leading to progressive distortion and dysfunction of liver architecture (Christidis, 2001). Fibrosis itself causes no symptoms but can lead to portal hypertension, hepatocyte ischemia or cirrhosis (Levison *et al.,* 1982). Kupffer cells, injured hepatocytes, platelets, and

leukocytes aggregate, releasing reactive O_2 species and inflammatory mediators which accelerate fibrosis.

2.4.3.3 *Hepatic cirrhosis*

Cirrhosis is a leading cause of death worldwide (Bosch *et al.,* 2008, Krige *et al.,* 2006). Cirrhosis of the liver is the terminal sequel of prolonged repeated injury to the hepatic parenchyma (Fang *et al.,* 2003). It may be due to alcoholic liver disease or viral hepatitis (Fang *et al.,* 2003). Cirrhosis is fibrosis that progresses to produce diffuse disorganization of normal hepatic structure, characterized by regenerative nodules surrounded by broad bands of fibrotic tissue (Brandão *et al.,* 2006). The liver is misshapen, nodular and shrunken weighing less than 1 kg in some extreme cases of cirrhosis. Cirrhotic patients have some evidence of hepatic cell dysfunction, including jaundice, anorexia, easy bruising and fatigue. The loss of normal liver tissue slows the processing of nutrients, hormones, drugs, and toxins by the liver. Also slowed is production of proteins and other substances made by the liver. In response to injury, growth regulators induce hepatocellular hyperplasia and arterial growth. Angiogenesis produces new vessels through which blood flow becomes distorted and along with compression of hepatic venules by regenerating nodules contributes to portal hypertension (Bosch *et al.,* 2008). Terminal consequences of liver cell necrosis are the accumulation of ammonia resulting in encephalopathy, liver failure and ascites (Rothuizen, 2009). Therapy of underlying liver disease is primarily supportive and no specific therapy can be instituted, other than liver transplantation.

2.4.3.4 *Fatty liver*

Fatty liver is an excessive accumulation of fat especially triglyceride inside hepatocytes, the most common liver response to injury (Adachi *et al.,* 2005). The most common causes of

fatty liver are alcoholism, obesity, diabetes, and elevated serum triglyceride levels. Other causes include malnutrition, hereditary disorders of metabolism and drugs (such as corticosteroids, tetracycline and aspirin. The mechanism by which these diseases or factors cause fat to accumulate within liver cells is not known (Sanyal, 2005). One possible explanation is these factors slow the rate at which fat is metabolized and excreted by the body. The resulting buildup of fat within the body is then stored inside the liver cells. In some cases fatty liver progresses to scarring (fibrosis) and cirrhosis, because of underlying inflammation (Friedman 2007).

2.4.3.5 *Hepatocellular carcinoma (HCC)*

Hepatitis B infection is strongly linked to the prevalence of hepatocellular carcinoma (HCC) (Kumada *et al.,* 2010). Also HCC, very often occurs on a background of cirrhosis (Brancatelli *et al.,* 2003). In many parts of the Asia, particularly in areas where viral hepatitis is common, primary cancers of the liver can represent up to 40% of all reported malignancies (Malcom, 2005). However they are extremely uncommon in Western countries and represent around 1% of all reported cancers (Perilongo *et al.,* 1990). Microscopically, HCC includes a well differentiated form with cells that are recognizably hepatocyte in origin. As the tumor becomes more anaplastic, the liver cells can be bizarre and often sufficiently undifferentiated to become spindle. The tumor shows a distinct tendency to invade vascular channels (Nagase *et al.,* 2000).

Hepatocellular necrosis occurs under a wide range of pathological conditions. In most cases, toxic cell death takes place over a finite span of time, accompanied by homeostatic counterresponses that are varied and complex. The present strategies for discovering critical steps in cell death recognize that (1) different types of injuries produce similar morphologic changes that precede killing in widely varied cell types, and that (2) lethal events are likely to involve one or more compartmentalized functions that are common to most cells.

Investigations of the lysosomes, plasma membrane, endoplasmic reticulum, cytoplasm, mitochondrion, and nucleus have greatly advanced our understanding of acute hepatocellular injury.

2.4.4 Subcellular responses of hepatocytes to injury

If hepatocytes are not significantly injured they may undergo proliferation - replication to replace dead cells or hypertrophy - enlargement of organelles produces enlargement of cells probably to increase function of individual hepatocytes (Colman *et al.*, 1983).

2.4.4.1 Lysosomes (heterophagy; autophagy)

The protection of cells from the activity of acid hydrolases by inclusion of the enzymes within lysosomes was first described by de Duve *et al.,* 1955. Since then it has been shown that rupture of the lysosomal membranes causes extensive damage to cell contents and that in the advanced stages of hepatocellular necrosis these enzymes are released into the circulation. Thus Slater and Greenbaum, 1965 found increased serum acid phosphatase activity hours after the oral administration of the hepatotoxin carbon tetrachloride to rats. Liver ischaemia and hypoxia increases the activity of plasma lysosomal enzymes (Grek *et al.,* 2003). In acute and chronic liver injury, many changes occur in the metabolism of lipids, one of them being unusually high levels of tissue free fatty acids (Mavrelis *et al.,* 1983). These fatty acids cause translocation of proapoptotic proteins into lysosomes (Feldstein *et al.,* 2006). This causes lysosomal destabilisation with release of lysosomal enzymes into the cytosol. Lysosomal destabilisation results in NFκB dependent TNF-α expression, which promotes triglyceride accumulation and hepatic steatosis (Boya *et al.,* 2003). Moreover, TNF-α can induce further lysosomal destabilisation and cathepsin B dependent apoptosis in a feed forward loop that exacerbates liver damage (Guicciardi & Gores, 2005).

2.4.4.2 Smooth ER (induction)

In a type of liver injury caused by drugs, many ultrastructural changes take place. Alcohol is an inducer of smooth endoplasmic reticulum (Rubin *et al.,* 1968). Thus, there is likely to be expansion of this membrane system. Expansion of the SER results in enlargement of the liver cells and relative clearing of the cytoplasm, producing a picture very much like cellular swelling due to increased water and electrolytes (Dombrowski *et al.,* 2000).

2.4.4.3 Mitochondria (number, size and shape)

Oxidative stress and mitochondrial injury play a role in the mechanisms of liver injury in viral infection (Choi & Ou, 2006). Liver tissue from virus-infected patients shows evidence of glutathione depletion, morphologic changes in mitochondria, and the presence of lipid peroxide-protein adducts (Zocco *et al.,* 2005). The viral core protein alters mitochondrial function and results directly in an increase in the cellular abundance of ROS with consequent increases in cellular lipid peroxidation. Mitochondrial reactive oxygen species production is induced by hepatitis virus core (Otani *et al.,* 2005) resulting in a reduction of mitochondrial antioxidant capacity and sensitivity to oxidants and TNF α. Alcohol abuse in hepatitis further depletes mitochondrial reduced glutathione, which exacerbates oxidative stress and causes cell death (Li *et al.,* 2007). Ethanol also produces mitochondrial structural abnormalities and decreases adenosine triphosphate synthesis in hepatic mitochondria (Otani et *al* 2005). It increases electron flux and ROS generation through the mitochondrial electron transport chain. All these effects lead to impaired mitochondrial function and an inability to cope with further oxidative insults. Viral core protein interacts directly with mitochondria where it causes oxidation of the glutathione pool, inhibition of complex I activity, increased reactive oxygen species production and enhanced sensitivity to oxidant-induced cell death (Korenaga *et al.,* 2005).

2.4.4.4 **Cytoskeleton**

The hepatic cytoskeleton is not an inert, rigid structure but that it is labile and in a state of continuous turnover (Lemasters *et al.,* 1983). There are several reports of surface bleb formation or apoptosis following various types of toxic and metabolic injury to tissues including liver (Lemasters, 1998). Hepatic injuries are characterized by prominent alterations of the hepatocellular cell surface (Gores *et al.,* 1990). The alterations include bleb formation on the sinusoidal surfaces of injured hepatocytes, loss of microvilli and disruption of microfilamentous structures of the cytoplasm. Hepatic enzymes may be released from viable tissues by shedding of cell surface blebs that are mostly cytosolic in origin into the circulation resulting in their appearance in the blood after liver injury (Thurman *et al.,* 1984). Enzyme release from injured liver tissue occurs even in the absence of outright hepatic necrosis. In mild or moderate cellular injury, cytosolic enzymes such as glutamate-pyruvate transaminase and lactate dehydrogenase can be released selectively but larger organelles such as mitochondria are excluded (Morales-Gonzalez *et al.,* 1999). In severe liver injury leading to necrosis, both mitochondrial and cytosolic enzymes appear in the blood (Solter, 2005). This occurs through a generalized breakdown of the plasma membrane permeability barrier leading to indiscriminate release of cellular contents.

2.4.4.5 **Nucleus**

Accumulating knowledge about two distinct modes of cell death, necrosis and apoptosis, indicates that loss of Ca2+ regulation and subsequent damage to DNA may be critical steps in lethal damage to liver cells (Ray *et al.,* 1991). Host genetic factors are more important than environmental factors in determining the severity of liver diseases (Zeng *et al.,* 2008). Liver injury is typically characterized by lipid peroxidation in the nuclear fraction along with mitochondrial fraction. The lipid peroxidation in turn is considered to be closely

related to the induction of liver cell death and to the production of alterations in DNA. In liver cell necrosis, hydropic changes and cytoplasmic swelling takes place. In the enlarged cells nuclear changes become more prominent. Liver cell shows necrotic nuclear shrinkage, absence or loss of normal structural organization into nuclei, granular chromatin and extreme clumping of nuclear components (Corcoran & Ray, 1992).

MATERIALS & METHODS

3. MATERIALS AND METHODS

3.1 Chemicals

Taurine, tetraethoxy propane and D-GalN were obtained from M/s. Sigma Chemical Company, St. Louis. MO, USA. All the other chemicals used were of analytical grade.

3.2 Animals

Adult male Wistar strain albino rats, weighing 100-120g were selected for the study. The animals were housed individually in polyurethane cages under hygienic conditions and maintained at normal room temperature. The animals were allowed food and water *ad libitum.* The experiment was carried out according to the guidelines of Committee for the Purpose of Control and Supervision of experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethics Committee.

3.3 Induction of fulminant hepatic failure

Fulminant hepatic failure was induced in experimental rats by injecting D-GalN (500 mg/100g body weight/day), i.p. for 2 days **(**Anandan *et al.,* 1999). The quantity of taurine was decided after preliminary experiments using 25, 50, 75, 100, 150 and 200mg of taurine/kg body weight.

3.4 Experimental protocol

Seven days after acclimatization, the animals were divided into four groups of 6 rats each.

1. Group I was taken as the control fed on commercial feed.
2. Group II was fed on commercial feed with added taurine (100mg/kg body weight) to see if taurine by itself caused any undesirable changes.
3. Group III was fed on commercial feed alone. After 30 days feeding, rats were given i.p. injection of D-GalN (500mg/100g body weight/day for 2 days).
4. Group IV was fed on commercial feed with added taurine and after 30 days they were given i.p.injection of D-GalN to see whether unlike in Group III the added taurine could prevent or lessen the adverse effects of D-GalN.

At the end of the experimental period, i.e., 24h after last injection of D-GalN, the experimental animals were sacrificed, blood was collected using sodium citrate as

anticoagulant and the plasma separated was used for assay of various biochemical parameters. The liver tissue was excised immediately and washed with chilled isotonic saline. One portion of tissue was fixed in 10% buffered formalin for histopathological observations. Accurately weighed liver tissue was homogenized in ice-cold 0.1 M Tris-HCl buffer, pH 7.2 and centrifuged. The supernatant was used for further biochemical analyses. For each set of anlyses feeding trials with taurine were conducted at different times using four separate sets of albino rats

3.4.1 Histopathology procedure

After ether anesthesia, the rat's liver (Wistar albino rats) was dissected by cutting on the ventral side. 2 – 3 mm. of the liver tissue was fixed in neutral buffered formalin (10% formaldehyde in Phosphate buffered saline) over night. After fixation, the tissues were immersed in 70% isopropyl alcohol for 3 hours and then in each ascending strength (80%, 90%, 100% isopropyl alcohol) for 2 hours each. The amount of alcohol used was 15 times of the size of the tissue. Then the tissues were dipped in acetone for a period of 1 – 2 h with periodical shaking. After removing the acetone, xylene was added to check for the appearance of milkiness. If milkiness appears the dehydration procedure was repeated. The dehydrated tissue was impregnated in paraffin wax (m.p. = 56ºC) for a period of 1h at 58 – 60 ºC. The molten paraffin was poured into L-block along with the tissues and allowed it to become hard. The tissue was sectioned into very thin (2–8 or 5 – 10 µm) sections using a microtome. The tissue was mounted on the slides with Mayer's albumin solution (a mixture of equal parts of egg white and glycerin, beaten and filtered with the addition of 1% sodium salicylate) and kept in warm oven for 2 h at 60ºC. Slides containing paraffin sections were placed on a slide holder. Slides were deparaffinized with Xylene for 20 – 30 minutes and the excess xylene was blotted. The tissue was rehydrated successively with 100%, 90%, and 80% isopropyl alcohol for 2 – 3 min. each and was put into water for 3 min. The excess water was blotted and the tissue was put into Hematoxylin stain for 1 – 2 min. It was removed from Hematoxylin stain and then again put into tap water for 1 – 2 min. The slides containing tissue sections were dipped into 1N HCl followed by Scott's water (Sodium Bicarbonate 3.5 g, Magnesium sulphate 20 g, distilled water 1 litre) for 1 min each. The tissue was immersed in Eosin stain for 30 secs. The tissue was dehydrated successively with 80%, 90%, 100% isopropyl alcohol and finally with Xylene for 20 – 30 min. Coverslip was placed on the slides

using one drop of DPX, taking care to leave no bubbles and was dried overnight to make the permanent slide.

3.5 Diagnostic marker enzymes

3.5.1 Assay of alanine aminotransferase (EC 2.6.1.2)

The activity of alanine aminotransferase (ALT) was determined by the method of Mohur & Cook (1957).

Reagents

1. Buffered substrate solution (0.1 M phosphate buffer, pH 7.4, 0.2 M DL-alanine, 2.0 mM 2-oxoglutarate): 1.5g dipotassium hydrogen phosphate, 0.2g potassium dihydrogen phosphate, 0.03g 2-oxoglutaric acid and 1.78g DL-alanine were dissolved in distilled water. The pH was adjusted to 7.4 with 1N NaOH and made up to 100 ml.
2. 20 mg 2,4 dinitrophenyl hydrazine (DNPH) in 100 ml of hot 1N hydrochloric acid.
3. 0.4N Sodium hydroxide.
4. Standard pyruvic acid: 12.5mg of sodium pyruvate was dissolved in 10 ml of distilled water.10 ml of this was diluted to 100 ml with distilled water and this was prepared freshly for the calibration curve.

Procedure

To 1.0 ml of the buffered substrate, 0.1 ml sample was added and incubated at 37˚C for 30 min. The reaction was arrested by adding 1.0 ml of DNPH and left aside for 20 min at room temperature. Color developed by the addition of 10 ml of 0.4N NaOH was read at 540nm in a Shimadzu UV spectrophotometer against the reagent blank. The enzyme activity was expressed as µmoles of pyruvate liberated /hr/l (plasma).

3.5.2 Assay of aspartate aminotransferase (EC 2.6.1.1)

The aspartate aminotransferase (AST) activity was assayed by the method of Mohur & Cook (1957).

Reagents

1. Phosphate buffer: 0.15M, pH 7.5
2. Substrate: 300mg of L-aspartic acid and 50mg of α-ketoglutaric acid were dissolved in 20-30 ml of the phosphate buffer and added 10% sodium hydroxide to bring the pH to 7.5 and was made up to 100 ml with phosphate buffer.
3. 2,4-dinitro phenyl hydrazine (DNPH) reagent: Dissolved 200mg of DNPH in 85 ml of concentrated hydrochloric acid and made up to a liter with distilled water.
4. 0.4 N sodium hydroxide.
5. Standard pyruvic acid: 12.5mg of sodium pyruvate was dissolved in 10 ml of distilled water.10 ml of this was diluted to 100 ml with distilled water and this was prepared freshly for the calibration curve.

Procedure

To 1.0 ml of the buffered substrate, 0.1 ml of the sample was added and incubated for one hour at 37oC. Then 1.0 ml of DNPH reagent was added and left for 20 min. At the end of incubation, 10 ml of 0.4 N NaOH was added and the colour developed was estimated by reading OD at 540nm in a Shimadzu UV spectrometer after 10 min. The standards were also treated similarly. The enzyme activity was expressed as µmoles of pyruvate liberated /hr/l (plasma).

3.5.3 Assay of lactate dehydrogenase (EC 1.1.1.27):

The lactate dehydrogenase (LDH) activity was assayed according to the method of King (1965) with slight modification. The amount of pyruvate formed in the forward reaction was measured colorimetrically

Reagents

1. 0.1M glycine buffer: 7.5g of glycine and 5.88g of sodium chloride were dissolved in one liter of distilled water.
2. Buffered substrate: 2.76g of lithium lactate was dissolved in 125 ml of glycine buffer containing 75 ml of 0.1 N sodium hydroxide to adjust the pH 10. This was prepared just prior to use.
3. 0.4N Sodium hydroxide.

4. 5.0mg of NAD+ was dissolved in 1.0 ml of distilled water. This was prepared just before use.
5. 2,4-dinitrophenyl hydrazine (DNPH): 200mg of DNPH was dissolved in one litre of 1N HCl.
6. Standard pyruvate solution: 12.5mg of sodium pyruvate was dissolved in 100 ml of buffered substrate.

Procedure

To 1.0 ml of the buffered substrate, 0.2 ml of the sample was added and the tubes were incubated at 37ºC for 15 min. After adding 0.2 ml of NAD^+ solution, the incubation was continued for 30 min and then 1.0 ml of DNPH reagent was added. And the tubes were incubated at 37ºC for 15 min. Then 7.0 ml of 0.4N NaOH was added and the colour developed was measured at 540nm in a Shimadzu UV spectrophotometer against the reagent blank. Suitable aliquots of the standards were also treated in the same manner. The enzyme activity was expressed as µmoles of pyruvate liberated /hr/l (plasma).

3.5.4 Gamma glutamyl transferase:

Principle of the assay is based on the rate of increase in absorbance due to release of p-nitroanaline and is measured at 405nm.

$$\text{Gamma-glutamyl-p-nitroanilide} + \text{glycylglycine} \xrightarrow{\gamma\text{- GT}} \text{gammaglutamyl-glycylglycine} + \text{p – nitroaniline}$$

Reagents:

1. 0.05M amino-2-m3thyl propan-1-3 diol buffer pH 9.3: 2.63 g AMP is dissolved in distilled water and pH adjusted with 1M HCl and diluted to 500ml. It was refrigerated.
2. Substrate: 12.05mg L-gamma glutamyl p-nitroanilide, 69.4mg glycyl glycine and 21.3 mg $MgCl_2.6H_2O$ in 10 ml buffer at 40ºC with constant stirring. pH adjusted to 8.2 and stored at room temperature.

Procedure:

In a quartz tube, 3 ml of substrate was taken and equilibriated at 25ºC. ΔA/min was monitored. 0.15 ml of tissue extract was added. The rate of increase in absorbance at 405 nm was recorded for 5 mins.

Calculation

$$\text{u/mg material} = \frac{\Delta A_{405}/\text{min} \times 3.15 \times \text{dilution}}{9.62 \times 0.15 \times \text{mg enzyme/ml original solution}}$$

3.5.5 Assay of alkaline phosphatase (EC 3.1.3.1):

Alkaline phosphatase was assayed by the method of King (1965) using disodium phenyl phosphate as the substrate.

Reagents

1. Carbonate- bicarbonate buffer:0.1M pH 10.0
2. Substrate: 0.01 M disodium phenyl phosphate solution.
3. Folin's phenol reagent: This was diluted 1:2 with double distilled water before use.
4. Sodium carbonate :15%
5. Magnesium chloride :0.1M
6. Standard phenol solution: A solution of distilled crystalline phenol in water, containing 50 µg/ ml was prepared.

Procedure

The incubation mixture contained the following components in a final volume of 3.0-ml.1.5 ml of carbonate- bicarbonate buffer, 1.0 ml of substrate and 0.1 ml of magnesium chloride and requisite amount of the enzyme source (0.2 ml serum). The reaction mixture was incubated at 370 C for 15 min. The reaction was terminated by the addition of 1.0 ml of Folin's phenol reagent. If turbidity appeared, the tubes were centrifuged. Controls without enzyme sources were also incubated and the enzyme source was added after the addition of Folin's phenol reagent. The 1.0 ml of 15% sodium carbonate solution was added and incubated for a further 10 min at 370 C. The blue colour developed was read at 640 nm against a blank. The standards were also treated similarly.

The activity of the enzyme is expressed as µmoles of phenol liberated/ hr/l (plasma); µ moles of phenol liberated/ mg protein (tissue).

3.5.6 Assay of acid phosphatase (EC 3.1.3.2):

Acid phosphatase was assayed by the method of King (1965) using disodium phenyl phosphate as the substrate.

Reagents

1. Citrate buffer :0.1 M pH 4.9
2. Substrate : 0.01 M Disodium phenyl phosphate solution.
3. Folin's phenol reagent: This was diluted 1:2 with double distilled water before use.
4. Sodium carbonate: 15%
5. Standard phenol solution: A solution of distilled crystalline phenol in water, containing 50µg /ml was prepared.

Procedure

The incubation mixture contained the following components in a final volume of 3.0 ml. 1.5 ml of citrate buffer, 1.0 ml of substrate, 0.3ml of distilled water and requisite amount of the enzyme source (0.2 ml serum). The reaction mixture was incubated at 370 C for 15 min. The reaction was terminated by the addition of 1.0 ml of Folin's phenol reagent. If turbidity appeared, the tubes were centrifuged. Controls without enzyme sources were also incubated and the enzyme source was added after the addition of Folin's phenol reagent. 1.0 ml of 15% sodium carbonate solution was added and incubated for a further 10 min at 370 C. The blue colour developed was read at 640 nm against a blank. The standards were also treated similarly.

The activity of the enzyme is expressed as µmoles of phenol liberated/ hr/l (plasma); µmoles of phenol liberated/ mg protein (tissue).

3.6 Serum bilirubin

Principle

Conjugated (direct) bilirubin in serum is coupled with diazotised sulphanilic acid to form a red coloured compound. Ascorbic acid is used to stop the coupling reaction, and to eliminate

interference by haemoglobin. Caffeine benzoate solution is used to split the unconjugated bilirubin protein complex releasing the bilirubin so that it can react with diazotised sulphanilic acid. The tartrate buffer makes the mixture alkaline and converts the red acid bilirubin to a green coloured compound which shows peak absorbance at 607 nm. At this wavelength the absorbance due to haemoglobin or carotene is minimal.

Reagents

1. Caffeine-benzoate: 100g caffeine sodium benzoate and 25 g sodium benzoate were dissolved together in 800 ml of distilled water. The solution was heated to 60^0C and 125g of hydrated sodium acetate and 1g of EDTA were added. The solution was mixed to dissolve and made up to 1 litre with distilled water. The solution was filtered. It is stored at room temperature (25-30^0C) which is stable for 6 months.
2. Sulphanilic acid: 5 g sulphanilic acid was dissolved in 500ml distilled water with heating. 15ml of conc. HCl was added and cooled and made up to 1 litre. It is stored at room temperature (25-30^0C) which is stable for 6 months.
3. Sodium nitrite: 500 mg sodium nitrite was dissolved in about 80ml distilled water and then made up to 100 ml. Stored at 2-8^0C. Prepared fresh once a month.
4. Diazo reagent: 10ml sulphanilic acid was mixed with 0.25ml sodium nitrite. The solution is stable for approximately 3 hours at room temperature (25-30^0C) and 24 hours at 2-8^0C.
5. Alkaline tartrate: 100g NaOH and 350 g sodium potassium tartrate were dissolved in distilled water and made up to 1 litre. It is stored at room temperature (25-30^0C) which is stable for 6 months.
6. Ascorbic acid (4 g/dl): 200 mg of ascorbic acid was dissolved in 5ml of distilled water. This solution must be freshly prepared each day.

Procedure

Reagents, standards and test samples were added in the order indicated into appropriately labelled tubes:

	Standard Blank	**Std**	**Test/QC Blank**	**Test/QC Direct**	**Test/QC Total**
Distilled water (ml)	0.8	0.8	0.8	0.8	0.8
Bilirubin Std (ml)	0.2	0.2	-	-	-

Test sample /QC (ml)	-	-	0.2	0.2	0.2
Diazo reagent (ml)	-	0.5	-	0.5	0.5
Caffeine sodium benzoate	-	2.0	-	-	2.0
Mixed and waited for 10 min at room temperature (25-30^{0}C)					
Ascorbic (ml)	0.1	0.1	0.1	0 .1	0.1
Diazo reagent (ml)	0.5	-	0.5	-	-
Caffeine sodium benzoate (ml)	2.0	-	2.0	2.0	-
Alkaline Tartrate (ml)	1.5	1.5	1.5	1.5	1.5

Tubes were mixed well. Spectrophotometer was set to zero with distilled water at 607nm and the absorbance was read in the order of assay tubes mentioned in the Table. The amount present in samples was calculated by regression obtained after calibration

3.7 Prothrombin time

1. The prothrombin time is most commonly measured using blood plasma.
2. Blood is drawn into a test tube containing liquid citrate, which acts as an anticoagulant by binding the calcium in a sample.
3. The blood is mixed, then centrifuged to separate blood cells from plasma.
4. The plasma is analyzed on an automated instrument at 37°C, which takes a sample of the plasma.
5. An excess of calcium is added (thereby reversing the effects of citrate), which enables the blood to clot again.
6. Tissue factor (also known as factor III) is added, and the time the sample takes to clot is measured optically.
7. The prothrombin ratio is the prothrombin time for a test, divided by the result for control plasma.

3.8 Mineral Analysis:

3.8.1 Determination of ash content:

About 2 g of accurately weighed moisture free sample from desiccators was taken in porecelain crucible. The sample was charred on burner for about half an hour and then placed in a temperature controlled furnace pre-heated to 550ºC for 4 hours or until complete ashing

took place. The crucible was cooled and weighed immediately. The process was repeated till the difference between the successive weighing was less than one milligram and the lowest weight was noted.

Calculation: Ash percentage by weight=W2-W/W1-W x 100

Where, W is weight of empty crucible

W1 is the weight of crucible with sample

W2 is the weight of crucible with ash

3.8.2 Determination of mineral content:

The ash obtained is dissolved in 100ml of 6N HCl quantitatively. The solution is appropriately diluted and aspirated in to the AAS for quantification. For the purpose of calibration, three sets of standard concentrations of each mineral are aspirated and the standard calibration curve is drawn. Following this the cell aspiration tubes are rinsed with distilled water. The samples are then aspirated and the reading is noted. The calculation is made based on the readings and dilution.

Value obtained in ppm= A(absorbance value) x volume made up/weight of sample taken for ashing

3.9 Arginase & blood urea

3.9.1 Arginase assay

Reagents

1. Saponin solution
2. Manganese chloride: 10%
3. Arginine: 0.02M
4. Sodium tungstate: 2.2%
5. Sulphuric acid: 0.15N
6. Phosphoric – sulphuric acid mixture
7. Diacetyl monoxime: 2%

Procedure

0.02ml tissue extract and 0.5 ml saponin solution were incubated at 37ºC for 10 min. 0.02ml of 10% $MnCl_2$ was added and incubated for further 10 mins. 0.2ml of 0.02M arginine was added and incubated for 20 mins. 1 ml of tungstic acid (2.2% sodium tungstate mixed

with 0.15 N sulphuric acid) and 0.26 ml of 0.1M HCl were added. The above mixture is centrifuged and 1 ml of the supernatant was taken for urea estimation. Urea estimation described below. 1 unit enzyme activity is defined as the amount of enzyme required to produce 1µmol urea per minute at 37ºC. In tissue it is expressed as units/g tissue and in serum as ng/ml.

3.9.2 Estimation of urea

Principle

Urea reacts directly with diacetyl monoxime under strong acidic conditions to give a yellow condensation product. The reaction is intensified by the presence of ferric ions and thiosemicarbazide. The intense red colour formed is measured at 540nm.

Reagents

1. Stock acid reagent: 1.0g of ferric chloride hexahydrate was dissolved in 30 ml of distilled water. 20 ml orthophosphoric acid was added and mixed. Stored in a brown bottle at room temperature (25-35^{0}C) which is stable for 6 months.
2. Mixed acid reagent: 100 ml of Conc. H_2S0_4 was added slowly to 400 ml distilled water taken in a 1-litre flat-bottom conical flask kept in an icecold waterbath. Mixed well and 0.3ml of stock acid reagent was added. Mixed and stored in a brown bottle at room temperature (25-35^{0}C) which is stable for 6 months.
3. Stock colour reagent – A: 2g diacetyl monoxime was dissolved in distilled water and the volume was made up to 100 ml in a volumetric flask. Stored in a brown bottle at room temperature (25-35^{0}C) which is stable for 6 months.
4. Stock colour reagent - 0.5 g thiosemicarbazide was dissolved in distilled water and made up to 100 ml in a volumetric flask. Stored in a brown bottle at room temperature (25-35^{0}C) which is stable for 6 months.
5. Mixed colour reagent: 35 ml of stock colour reagent A was mixed with 35 ml of stock colour reagent B and made up to 500 ml with distilled water. Stored in a brown bottle at room temperature (25-35^{0}C) which is stable for 6 months.
6. Stock urea standard: 1.0g of analytical-grade urea was dissolved in 100ml of benzoic acid (1g/dl). A 100ml of volumetric flask was used for preparing this. Stored in brown bottle at 25-35^{0}C which is stable for 6 months.

7. Working standard 50mg/dl: 5.0ml of stock urea standard was diluted to 100 ml with benzoic acid. Stored in a brown bottle at room temperature (25-35^{0}C) which is stable for 6 months.

Procedure

Dilution of Standards (S1-S3), Test solutions are prepared as follows: The following are pipetted into appropriately labelled tubes and mixed well.

	S1	**S2**	**S3**	**Test**
Distilled Water (ml)	1.9	1.8	1.7	1.9
50 mg/dl Urea (ml)	0.1	0.2	0.3	-
Test sample (ml)	-	-	-	0.1

The colour reagent is prepared fresh at the time of analysis by mixing distilled water, mixed acid reagent and mixed colour reagent in the ratio 1:1:1. Following are taken.

	Blank	**S1**	**S2**	**S3**	**Test**
Colour reagent (ml)	3.1	3.0	3.0	3.0	3.0
Respective diluted standard ml)	-	0.1	0.1	0.1	-
Diluted test (ml)	-	-	-	-	0.1

All tubes were mixed well. Kept them in a boiling water-bath for 15 min. Removed from waterbath and cooled the tubes for 5 min. The spectrophometer was set to zero with blank at 540nm and the absorbance of the other tubes was measured. Standards:

S1 = 50 mg/dl

S2 = 100 mg/dl

S3 = 150 mg/dl

The absorbance values of standards was plotted against their respective concentrations. The measurable range with this graph is from 10 to 150 mg/dl. A calibration graph was constructed whenever a new set of reagents were prepared. Absorbance values of test were plotted on the calibration graph and the concentrations of test samples were determined.

3.10 Glucose metabolism

3.10.1 Blood glucose

Blood glucose was estimated by the method of Sasaki & Matsui, (1972) using o-toluidine reagent.

Reagents

1. Trichloro acetic acid (TCA) : 10%
2. o-Toluidine reagent:

12.5 g of thiourea and 12.0 g of boric acid were dissolved in 50 ml of distilled water by heating over a mild flame. Exactly 75 ml of redistilled o-toluidine and 375 ml acetic acid were mixed wth thiourea- boric acid mixture and the total volume was made up to 500 ml distilled water. The reagent was left in a refrigerator overnight and filtered.

3. Standard glucose solution

10 mg of pure glucose was dissolved in 100 ml of 0.2% of boric acid in water.

Procedure

0.12 ml of blood was mixed with 1.9 ml of TCA solution to precipitate the protein and centrifuged. 1.0 ml of supernatant was mixed with 4.0 ml of o-toluidine reagent and was kept in a boiling water bath for 15 min. The green colour was read at 600 nm in a UV spectrophotometer. A set of standard glucose solution were also treated similarly. Blood sugar level was arrived at by the comparision with the standard curve.

The values are expressed as mg per dl in blood.

3.10.2 Liver glycogen & glycogen phosphorylase

Tissue glycogen

Tissue glycogen was estimated by the method of Morales *et al.,* (1973).

Reagents

1. Potassium hydroxide solution :30%
2. Absolute ethanol
3. Anthron reagent : 0.2% solution in concentrated sulphuric acid, prepared just prior to use.
4. Standard glucose solution : 100mg of glucose was dissolved in 100ml of distilled water.

Procedure

A weighed amount of dried deffated tissue from each organ was subjected to alkali digestion by heating with 30% potassium hydroxide solution in a water bath for 20 min. The tubes were cooled and to this was added 5.0 ml of absolute ethanol to precipitate glycogen. The tubes were placed in a freezer overnight. The precipitated glycogen was collected by centrifugation at 2000g for 20 min. The glycogen was dissolved in water, reprecipitated with ethanol and centrifuged. The final precipitate was dissolved in water and heated for 5 min. Aliquots of glycogen solution were taken up after suitable dilution.Cooled in ice and mixed well with 4 ml of Anthrone reagent. The tubes were covered with glass marbles and heated in a boiling water bath for 10 min.The colour developed was read at 640 nm in a UV spectrophotometer. Standard glucose solution containing 20-100 mg were also subjected to the same procedure and a standard curve was obtained. The values are expressed as mg/g wet tissues.

Glycogen phosphorylase (EC 2.4.1.1)

Glycogen phosphorylase activity was determined by measuring the rate of liberation of inorganic phosphorous from glucose-1-phosphate in the presence of glycogen.

Reagents

1. Substrate
   0.05M glucose-1-phosphate solution containing 5.7 mg glycogen per ml and 0.05 M sodium fluoride, was prepared in distilled water. The pH was adjusted to 6.1 using dilute hydrochloric acid.
2. 5’ AMP : 0.025M
3. TCA : 10%
4. Sodium fluoride : 0.1N

Enzyme source was diluted with ice cold 0.1M sodium fluoride solution. Dilution was carried to just before use.

Procedure

The reaction was started by the addition of 1.0 ml of substrate, 0.1 ml of 5’ AMP, 0.2 ml of sodium fluoride and 0.2 ml of enzyme. An aliquot (0.5ml) was taken from the reaction

mixture at zero time and after 10 min of incubation at 37^0C. 1.0 ml of 10% TCA solution was added to arrest reaction. The contents were mixed well, centrifuged and the liberated Pi in the supernatant was estimated by the method of Fiske & Subbarow (1925).

The enzyme activity is expressed as n moles of Pi liberated /min/mg protein.

3.10.3 Glycolytic enzymes

3.10.3.1 Assay of Hexokinase (EC 2.7.1.1)

Hexokinase was assayed by the method of Brankstrup *et al.,* (1957).

Reagents

1. Glucose solution :0.005M
2. ATP solution: 0.72M
3. Magnesium chloride solution : 0.05M
4. Dipotassium hydrogen phosphate : 0.0125M
5. Potassium chloride solution : 0.1M
6. Sodium fluoride solution : 0.5M
7. Tris HCl buffer (pH 8.0) : 0.01M

Procedure

The reaction mixture in a total volume of 5.0ml contained the following: 1.0ml of glucose solution, 0.5ml ATP solution, 0.1ml of Magnesium chloride, 0.4ml of Dipotassium hydrogen phosphate solution, 0.4ml of Potassium chloride, 0.1ml of Sodium fluoride solution and 2.5ml of Tris HCl buffer (pH 8.0).The mixture was pre-incubated at 37^0C for 5 min. The reaction was initiated by the addition of 2.0ml of tissue homogenate. 1.0 ml aliquot of the reaction mixture was taken immediately (zero time) to tubes containing 1.0ml of 10% TCA. A second aliquot was removed after 30 min of incubation at 37^0C. The precipitated protein was removed by centrifugation and the residual glucose in the supernatant was estimated by the o-toluidine method of Sasaki & Matsui (1972) as described previously. A reagent blank was run with each test. The difference between the two values gave the amount of glucose phosphorylated. The enzyme activity is expressed as n moles of glucose-6-phosphate formed/min/mg protein.

3.10.3.2 Glucose-6-phosphate dehydrogenase (EC 1.1.1.49)

Glucose-6-phosphate dehydrogenase activity was determined by the method of Eals & Krikman (1961).

Reagents

1. Tris-HCl buffer : 0.05 M, pH 7.5
2. Magnesium chloride : 1.0M
3. NADP : 1.0M solution
4. Phenazine methosulphate (PMS) : 0.005%
5. 2,6-dichloro phenol indophenols (DCPI) : 0.01%
6. Glucose-6-phosphate : 0.02M

Procedure

The incubation mixture in a total volume of 5.5 ml contained the following : 1.0 ml of Tris-HCl buffer, 0.5 ml of PMS, 0.4 ml of DCPI solution, 0.1 ml of magnesium chloride. 0.1 ml of NADP and a requisite amount of enzyme preparation. The mixture was allowed to stand at room temperature for 10 min to permit the oxidation of endogenous materials. The reaction was initiated by the addition of 0.5 ml of glucose-6-phosphate. The optical density was read at 640 nm against a water blank in a UV spectrophotometer. The enzyme activity is expressed as units/mg protein.

3.10.4 Gluconeogenetic enzymes

3.10.4.1 Glucose-6-phosphatase (EC 3.1.3.9)

Glucose-6-phosphatase was assayed according to the method of King (1965).

Reagents

1. Citrate Buffer : 0.1M, pH 6.5
2. Substrate : Glucose-6-phosphate, 0.10M
3. Ammonium molybdate
4. ANSA
5. TCA : 10%

Procedure

The incubation mixture in a total volume of 1 ml contained 0.3 ml of buffer, 0.5 ml of substrate and 0.2 ml of the enzyme. Incubation was carried out at 37^0C for 60 min. The reactions arrested by the addition of 1 ml of TCA and centrifuged. The phosphorus content of the supernatant was estimated by the method of Fiske & Subbarow (1925).

The enzyme activity is expressed as µ moles of Pi liberated/min/mg protein.

3.10.4.2 Fructose-1,6-bis phosphatase (EC 3.1.3.11)

Fructose-1, 6- diphosphatase was assayed by the method of Gancedo & Gancedo (1971).

Reagents

1. Tris-HCl buffer : 0.1M, pH 7.0
2. Substrate : Fructose-1,6-diphosphate 0.05M
3. $MgCl_2$: 0.1M
4. KCl : 0.1M
5. EDTA : 0.001M
6. TCA : 10%
7. Ammonium molybdate
8. ANSA

Procedure

The assay medium in a final volume of 2 ml contained 1.2ml of buffer 0.1 ml of substrate 0.25 ml of magnesium chloride, 0.1 ml of potassium chloride, 0.25 ml of EDTA and 0.1 ml of tissue homogenate. The incubation was carried out at 37^0C for 15 min. The reaction was terminated by the addition of 1 ml of 10% TCA. The suspension was centrifuged and the phosphorous content of the supernatant was estimated by the method of Fiske & Subbarow (1925).

The enzyme activity is expressed as n moles of Pi liberated /min/mg protein.

3.11 Protein, Amino acid and Glycoprotein Components.

3.11.1 Estimation of protein

The protein content in the sample was estimated by the method of Lowry *et al.,* (1951)

Reagents

1. Alkaline copper reagent
   Solution A: 2% sodium carbonate in 0.1 N sodium hydroxide solution.
   Solution B: 0.5% Copper sulfate in water.
   Solution C: 1% sodium potassium tartarate in water.
   50ml of solution A was mixed with 0.5 ml of solution B and 1ml of solution C just before use.
2. Folin's phenol reagent: Diluted 1:2 with double distilled water before use.
3. Standard bovine serum albumin (BSA): dissolved 100 mg of BSA in 100 ml of distilled water in a standard flask.10 ml of the stock was diluted to 100ml to get a working standard of 100µg/ml.

Procedure

Pipetted out 0.1ml of sample and standard BSA in the range of 20-100µg into test tubes and the total volume was made up to 1.0 ml with distilled water. The blank contained 1.0ml of distilled water. Exactly 4.5 ml of alkaline copper reagent was added to all the tubes and left at room temperature for 10 min after which was added 0.5 ml of Folin's phenol reagent. The blue colour developed was read after 20 min at 640 nm against the reagent blank, in a Shimadzu-UV-visible spectrophotometer.

The protein values are expressed as g/dl in plasma; mg /g in tissue.

3.11.2 Estimation of Amino Acids

Total amino acids and free amino acids in the serum and liver tissue were determined as per the procedure of Palmero *et al.,* (1992).

Reagents

1. HCl : 6N
2. HCl : 0.05M

3. Buffer A: Tri sodium citrate (58.8g) was dissolved in 2 L of double distilled water, add 210 ml ethanol of 99.5%, the pH was adjusted to 3.2 by adding 60% perchloric acid and made up to 3 L using double distilled water.
4. Buffer B: Tri sodium citrate, 58.8 g and boric acid, 12.4 g were dissolved in double distilled water, the pH was adjusted to 10 by adding 4N NaOH, and made up the volume to 1L using double distilled water.
5. Phthaladehyde (OPA) Buffer: 122.1 g of Na_2CO_3, 40.7 g of H_3BO_3 and 56.4 g of K_2SO_4 were dissolved in double distilled water and made up the volume to 3L.
6. Phthalaldehyde solution (OPA): 400 mg OPA, 7 ml ethanol, 1 ml of 2-Mercaptoethanol and 2ml of 30% w/v Brij-35 were dissolved in 500ml OPA buffer.
5. Sodium hypochlorite solution: 4% w/v Sodium hypochlorite in OPA buffer. ie., 0.3ml Sodium hypochlorite in100ml OPA buffer.

3.11.2.1 Total amino acids

Principle

The amount of each amino acid present within a given protein does not vary from molecule and can provide useful information about the nature of the protein molecule. In a typical analysis of the amino acid content of a protein, peptide bonds are broken by acid hydrolysis with 6N HCl at 110^0 C (24h) so that the released amino acids can be assayed. The amino acid tryptophan is not stable to acid digestion in the presence of even trace amounts of oxygen and is estimated separately by alkali digestion.

Sample preparation

Sample (100 mg tissue or 0.2 ml serum) was taken in a heat stable test tube. 10ml of 6NHCl was added and the tube was heat sealed after filling with pure nitrogen gas. The hydrolysis was carried out at 110^0C for 24 hrs. After the hydrolysis is over, the test tube was opened. The contents were quantitatively removed and filtered into a round bottom flask through Whatmann filter paper. No 42. The filter paper was washed 2-3 times with distilled water. The contents of the flask were flash evaporated to remove all traces of HCl, and the process was repeated for 2-3 times with distilled water. The residue was dissolved and made up to 10 ml with 0.05 M HCl.

HPLC Analysis

The sample prepared was filtered again through a membrane filter of 0.45µm and 20µl of this was injected into an amino acid analyzer (HPLC- LC 10 AS) equipped with cation exchange column packed with a strongly acidic cation exchange resin i.e., styrene di vinyl benzene co polymer with sulphinic group. The column used was Na type i.e., ISC- 07/S 1504 Na having a length of 19 cm and diameter 5mm.

The instrument was equipped with Shimadzu FL 6A fluorescence detector and Shimadzu CR 6A Chrompac recorder. The mobile phase of the system consisted of two buffers, Buffer A and buffer B. A gradient system was followed for the effective separation of amino acids. The oven temperature was maintained at 60^0 C. The total run was programmed for 62 min. The amino acid analysis was done by fluorescence detection after post–column derivatization with o-phthalaldehyde. In the case of proline and hydroxyl proline, imino group is converted to amino group with hypochlorite.

An amino acid standard (Sigma chemical Co., St. Lousis, USA) was run to calculate the concentration of amino acids in the sample. Calibration of equipment using standards was done before the start of analysis.

Quantification of amino acids

The standard and the sample were analyzed under identical conditions. The elution time of the amino acids of the sample was compared and identified with those of the standard. Quantification of amino acid was done by comparing the respective peak areas in the chromatogram of the sample and the standard. The amino acid content was calculated as follows:

g amino acid/ 100 g tissue =

$$\frac{\mu\text{mol of a.a in the injected vol} * \text{mol.wt} * \text{vol madeup} * 1000 * 100}{1000 * 1000 * 20 * \text{wt. of sample}}$$

The amount of each amino acid is expressed as g amino acid/ 100 g tissue and g amino acid/ dl serum.

3.11.2.2 Estimation of tryptophan

Tryptophan was estimated as per the method of Sastry and Tummuru (1985) after alkali hydrolysis of the sample using 5% sodium hydroxide at 110^0C for 24 hours. The 5-hydroxy furfural resulting from sucrose under acidic conditions formed a pale green coloured

condensation product with thioglycolic acid, which on treatment with tryptophan formed a pink coloured complex. The colour intensity is measured at 500nm.

Reagents

1. NaOH : 5%
2. HCl : 6N
3. Sucrose :2.5%
4. Thioglycolic acid : 0.6%
5. H_2SO_4 :50%
6. HCl : 0.1N
7. Tryptophan Standard: 10μg/ ml solution

Procedure

Sample was hydrolyzed with 10ml of 5% NaOH at 110^0C for 24 hours in a sealed tube with pure nitrogen. The hydrolysate was neutralized to pH 7.0 with 6N HCl using phenolphthalein indicator. The volume was made up to 25 ml with distilled water. The solution was then filtered through Whatman filter paper no.1 and filtrate was used for estimation.

The test tube containing 4 ml of 50% H_2SO_4, 0.1mlk of 2.5% sucrose and 0.1ml of 0.6% thioglycolic acid were added. These tubes were kept for 5 min in water bath at -50^0C and cooled. The sample was then added to the test tubes. A set of (0.1 to 0.8ml) standard tryptophan (10μg/ ml solution) was run in a similar way. The volume was made up to 5ml with 0.1N HCl and allowed to stand for 5 min for the development of colour. The absorbance was measured against a reagent blank at 500 nm in a spectrophotometer. Values expressed as mg tryptophan/100gm tissue.

3.11.3 Estimation of Hexose

Hexose was estimated by the method of Niebes (1972).

Reagents

1. Sulphuric acid: water mixture (3:2,v/v).
2. 800mg of orcinol dissolved in 50 ml of 1 N H2SO4.

3. Orcinol-sulphuric acid mixture: 1.0 ml of reagent (2) was mixed with 7.5 ml of reagent (1). This mixture was prepared fresh at the time of assay.
4. Standard hexose: Equal quantities of galactose and mannose were dissolved in water to give a concentration of 100 µg/ml.

Procedure

An aliquot of the delipidised sample was treated with 1.0 ml of 0.1 N NaOH. Blank contained 1.0 ml of 0.1N NaOH .The tubes were cooled by placing in an ice-bath and 8.5 ml of Orcinol-sulphuric acid mixture was added slowly and mixed well. The tubes were stoppered, incubated at 80^{o}C for 15 min in a water bath. Cooled and the color was allowed to develop in the dark for 25 min. The intensity was measured at 540 nm. Standard solutions containing 0.025 to 0.1 mg were treated similarly and hexose concentration was estimated. The concentration of hexose was expressed as mg/dl (plasma); mg/g (liver).

3.11.4 Estimation of hexosamine

Hexosamine was estimated by the method of Wagner (1979).

Reagents

1. Acetyl acetone reagent: 3.5% acetyl acetone in 1N Trisodium phosphate containing 0.5N potassium tetra borate (98: 2 V/V).
2. Ehrlich's reagent: 3.2g of P-dimethyl aminobenzaldehyde was dissolved in 30 ml of 1 N HCl and diluted to 210 ml with isopropanol.
3. Standard Hexosamine: Galactosamine hydrochloride solution containing 10 mg/100ml was prepared.

Procedure

An aliquot of the delipidised sample was hydrolyzed with 3N HCl in a boiling water bath for 4 hrs and neutralized. 0.8 ml of the neutral hydrolysate was mixed with 0.6 ml of acetyl acetone reagent. The mixture was heated in a boiling water bath for 30 min, cooled and 2.0 ml of Ehrlich's reagent was added. The contents of the tubes were mixed and the absorbance was measured at 535 nm. Standard hexosamine solution containing 20µg to 80µg was used for the preparation of standard curve.

Hexosamine was expressed as mg/dl (plasma); mg/g (liver).

3.11.5 Estimation of sialic acid.

Sialic acid was estimated by the method of Warren (1959).

Reagents

1. Sodium meta arsenite : A 10% solution was prepared in 0.5 M sodium sulphate in 0.1 N H_2SO_4.
2. Sodium meta periodate : 0.2M solution in 9 M phosphoric acid.
3. Thiobarbituric acid reagent : 0.6% solution was prepared in 0.5 M sodium sulphate
4. Acidified butanol : 5ml of con HCl in 95ml of n-butanol.
5. Standard sialic acid : 10mg of N-acetyl neuraminic acid was dissolved in 100ml of distilled water.
6. Working standard : 1ml of the stock was diluted to 10 ml using distilled water.

Procedure

An aliquot of the delipidised sample was hydrolyzed with 0.1 N H_2SO_4.at 80^0C for 1 hour. 0.2ml of the hydrolysate was mixed with 0.1ml of meta periodate and the solution was kept at room temperature for 20 minuts.1.0ml of sodium meta arsenite was added and shaken well so that the yellow brown colour disappeared. 3.0ml of thiobarbituric acid reagent was added and heated in a boiling water bath for 15 min. After cooling, 4.0 ml of acidified butanol was added, shaken well and the colour was extracted into butanol phase. The butanol phase was transferred to another set of tubes and the colour intensity was measured at 530 nm. The blank containing 0.2ml of 0.1 N H_2SO_4. and standard sialic acid solutions were treated similarly. Sialic acid is expressed as mg/g tissue.

3.12 Lipids

3.12.1 Extraction of total lipids

The lipid content of the tissues was estimated by the method of Folch *et al.*,(1957).

Reagents

Chloroform-methanol mixture (2:1 v/v)

Procedure

A weighed amount of the tissue was subjected to lipid extraction using chloroform-methanol mixture (2:1). The extraction was repeated twice with fresh aliquot of chloroform-methanol mixture. The lipid extracts were transferred to a separating funnel and added 20% of water into it and left overnight. Next day the lipid extracts were drained through filter paper containing anhydrous sodium sulphate and was collected in round bottom flask and was evaporated to dryness in a flash evaporator. The lipid in the round bottom flask was made up to 10 ml by using chloroform. From this 1.0 ml was taken into a pre-weighed vial and allowed to dry in warm temperature to constant weight and total lipid content were calculated from the difference in weight. Sample made up to 10 ml was used for the estimation of various lipid components viz., cholesterol (total and free), triglycerides, free fatty acids and phospholipids after evaporating the solvent in air at room temperature.

3.12.1.1 Estimation of total cholesterol

The total cholesterol present in plasma and liver was estimated according to method of Parekh and Jung (1970) with slight modifications.

Reagents

1. Standard cholesterol solution (stock): 1mg /ml in chloroform
2. Working standard: 1.0 ml of the stock was diluted to 10 ml with chloroform.
3. FeCl3 stock solution: 10g FeCl3 in 100 ml acetic acid.
4. FeCl3 - H2SO4 reagent: 2.0 ml of FeCl3 stock solution was diluted to 200 ml with conc. H2SO4.
5. 33% KOH (w / v): 10g of KOH was dissolved in 20 ml distilled water.
6. Alcoholic KOH solution: 6.0 ml of 33% KOH was made up to 100 ml with distilled ethanol. This solution is prepared fresh before use.

Procedure

1.0 ml of the lipid sample was taken into a 25 ml glass stoppered tube and evaporated off the chloroform. Added 5 ml of freshly prepared alcoholic KOH solution. The tubes were shaken well and incubated in a water bath at 370C for 55 min. After cooling to room temperature, added 10 ml of petroleum ether and inverted the tubes once to mix the contents. Then added 5.0 ml of distilled water and shaken the tubes vigorously for 1 min. Took 0.5-2 ml aliquots from the supernatant (petroleum ether) into test tubes. Evaporated the petroleum ether extract under nitrogen. To each of the sample as well as the standard tubes including the blank, added 3.0 ml of glacial acetic acid followed by 0.1ml -distilled water. Mixed the tubes thoroughly and added 2 ml of the FeCl3 - H2SO4 reagent to the sides of the test tubes. A brown ring was formed at the interface; tap the bottom of the tubes well to effect mixing and a light colour appeared which changed to an immense purple colour, which was measured in a Shimadzu-UV spectrophotometer at 560nm.

The amount of total cholesterol was expressed as mg/dl (plasma); mg/g (liver).

3.12.1.2 Estimation of triglycerides

The level of triglycerides in plasma and liver were determined by the method of Rice (1970) with slight modifications.

Reagents

1. Activated silicic acid.
2. Saponification reagent: 5.0g of potassium hydroxide was dissolved in 60 ml distilled water and 4.0 ml isopropanol.
3. Sodium metaperiodate reagent: To 77g of anhydrous ammonium acetate in 700 ml distilled water, added 60 ml glacial acetic acid and 650 mg of sodium metaperiodate and was dissolved and diluted to 1 litre with distilled water.
4. Acetyl acetone reagent: To 0.75 ml of acetyl acetone, 20 ml of isopropanol was added and mixed well.
5. Stock solution: 400mg of triolein was dissolved in 100 ml chloroform.
6. Working standard: 1.0 ml of the stock solution was diluted to 10 ml.

Procedure

0.2 ml of the lipid sample was taken into a test tube and evaporated off the chloroform, added 4.0 ml isopropanol. It was mixed well and added 0.4g of activated silicic acid. It was shaken in a vortex mixer for 15 min and centrifuged at 4000rpm for 5 min. To 2.0 ml of the supernatant and standards ranging from 20-100mg made up 2.0 ml with isopropanol, 0.6 ml of saponifying reagent was added and incubated at 60-70^{0}C for 15 min. After cooling, 1.0 ml sodium metaperiodate solution was added and mixed. To this, 5ml acetyl acetone was added, mixed and incubated at 50^{o}C for 30 min. After cooling, the colour was estimated by measuring OD at 405nm in a Shimadzu-UV spectrophotometer. The value of triglyceride in plasma was expressed as mg per dl and in tissue as mg per gm.

3.12.1.3 Estimation of free fatty acids

Free fatty acids in plasma and liver were estimated by the modified method of Horn and Menahan (1981).

Reagents

1. Activated silicic acid
2. Chloroform, heptane, methanol (CHM) solvent mixture: It was prepared by mixing chloroform, heptane and methanol in the ratio of 200:150:7(v/v)
3. Copper-triethanolamine solution: 50 ml of 0.1M copper nitrate and 50 ml of 2M triethanolamine were mixed with 33 g of sodium chloride. The pH of the solution was adjusted exactly to 8.1.
4. Diethyldithiocarbomate (DDC) solution: 0.1% DDC in butanol was prepared.
5. Standard Stock: A solution containing 2 mg per ml of palmitic acid was prepared in CHM solvent. For working standard, the stock was diluted 1:10 in CHM to give a concentration of 200µg per ml.

Procedure

To 1.0 ml of the lipid sample, 6.0 ml of CHM solvent and 200mg of silicic acid were added. The mixture was shaken well, centrifuged at 4000rpm for 5 min and 3.0 ml of the supernatant taken. Standard solution in the range of 25-100 µg were taken and made up to 3.0 ml with CHM solvent. The blank contained 3.0 ml of CHM solvent. To all these samples, 2.0 ml of copper triethanolamine solution was added and then mixed on a mechanical shaker for 10 min. The tubes were centrifuged at 4000rpm for 5 min. To the 2.0 ml of the supernatant taken, 1.0 ml of DDC solution was added and shaken well. The colour intensity was read

immediately at 430nm in a Shimadzu-UV spectrophotometer. Values were expressed as mg/dl plasma and mg/g wet tissue**.**

3.12.1.4 Estimation of phospholipids

Phospholipid content of plasma and liver was estimated by the method of Fiske and Subbarow (1925) as inorganic phosphorus liberated after Bartlette's perchloric acid digestion (Bartlette, 1959).

Reagents

1. Ammonium molybdate reagent: 2.5g of ammonium molybdate was dissolved in 100 ml of water.
2. Aminonapthosulfonic acid (ANSA): 0.5g of 1,2,4 aminonapthosulfonic acid was dissolved in 195 ml of 15% sodium metabisulfite and 50 ml of 20% sodium sulfite was added for complete solubilisation. The solution was filtered and stored in a brown bottle.
3. Stock standard solution: 351mg of potassium dihydrogen phosphate was accurately weighed, dissolved and made upto 100 ml with double distilled water to give a final concentration of 80 mg phosphorus per ml. Working standard: 1ml of the stock was diluted to 10 ml to give a conc. of 80µg phosphorus per ml.

Procedure

1 ml of the lipid sample was taken into a test tube and evaporated off chloroform. Added 0.5 ml of perchloric acid, the tubes were made up to 3.0 ml with double distilled water, and 1.0 ml of aliquot was taken. The tubes were made up to 4.0 ml with double distilled water. To all the tubes, 0.5 ml of ammonium molibdate reagent was added. After 10 min, added 0.5 ml of ANSA to all tubes. Aliquots of the standards and blank were carried through the same procedure. The blue colour developed was read after 20 min, at 620nm in a Shimadzu-UV spectrophotometer. The phospholipid content of plasma was expressed as mg per dl serum and same as per gm tissue.

3.12.2 Lipoprotein fractionation

Addition of heparin-manganous chloride to plasma caused the precipitation of VLDL and LDL.The supernatant represented the HDL fraction. n another aliquot of plasma, addition of sodium dodecyl sulphate resulted in aggregation of VLDL. The cholesterol content of each fraction was carried out in the following manner.

Total plasma cholesterol - (HDL+LDL) cholesterol = VLDL cholesterol

(HDL+LDL)- HDL = LDL

3.12.2.1 Estimation of high density lipoprotein fraction

Total HDL was separated by the method of Burstein et al (1972).

Reagents

Heparin-Manganous chloride reagent: 3.167gm of manganous chloride was added to 1.0 ml of heparin containing 20,000 units/ml. This was made up to 8.0 ml with water.

Procedure

2.0 ml of plasma was added to 0.09 ml of heparin-manganous chloride reagent and mixed well. The solution was allowed to stand at 4oC for 30 min. The supernatant represented HDL fraction. Aliquots were taken from HDL fraction for the estimation of cholesterol.

3.12.2.2 Estimation of low density lipoproteins

This differential analysis was made by the method of Burstein and Scholnick (1972) using sodium dodecyl sulphate.

Reagent

1. Sodium dodecyl sulphate: 10% in 0.15M sodium chloride pH 9.0

Procedure

To 1.0 ml of plasma, 0.75 ml of sodium dodecyl sulphate solution was added, which was taken in a ploy carbonate centrifuge tube. The contents were swirled briefly and packed for 2h in a water bath at 35^{o}C. The contents were centrifuged in a refrigerated centrifuge at

10,000g for 30 min. VLDL got aggregated as a pellicle at the top. The supernatant was a mixture containing HDL and LDL cholesterol was estimated in 0.05 ml aliquot of the supernatant as described above.

3.12.3 Estimation of triglyceride lipase (EC 3.1.1.3)

Lipase activity was assayed by a combination of methods of Schmidt et al (1974) with slight modifications. Tripalmitin was used as substrate. The liberated fatty acids were extracted using CHM solvent mixture and converted to their copper salts, which were determined colorimetrically using diethyldithiocarbomate.

Reagents

1. Substrate: 5g of Tripalmitin and 5g of gum arabic were mixed with 95 ml of 0.89% NaCl in a waring blender for 25 min and filtered.
2. Deoxycholate: 10mM aqueous solution.
3. Triethanolamine buffer: pH 8.5, 1.0 M solution.
4. Chloroform, heptane, methanol (CHM) solvent mixture: It was prepared by mixing chloroform, heptane and methanol in the ratio of 200:150:7 (v/v).
5. Copper-Triethanolamine solution: 50 ml of 0.1M copper nitrate and 50 ml of 2M Triethanolamine were mixed with 33g of sodium chloride. The pH of the solution was adjusted to 8.1.
6. Diethyldithiocarbomate (DDC) solution: 0.1% DDC in butanol was prepared.
7. Standard stock: solution containing 2 mg per ml of palmitic acid was prepared in CHM solvent. For working standard, the stock was diluted 1:10 in CHM to give a concentration of 200μg per ml.

Procedure

The incubation mixture was containing 30mM of the substrate, 0.5mM Deoxycholate and 0.5mM of Triethanolamine-buffer in a total volume of 1.0 ml. To this an aliquot of suitably diluted enzyme preparation was added and incubated at 30^{o}C for 30min with intermittent shaking. At the end of incubation period, the tubes were immersed in a boiling water bath for 1 min to arrest the enzyme reaction. Inactivated enzymes were obtained by heating were added to control tubes. 6.0 ml of CHM solvent were added. To all these tubes, 200mg of silicic acid were added. The mixture was shaken well, centrifuged at 4000rpm for 5 min and

3.0 ml of the supernatant was taken. Standard solution in the range of 25-100µg were taken and made up to 3.0 ml with CHM solvent. The blank contained 3.0 ml of CHM solvent. To all these samples, 2.0 ml of copper triethanolamine solution was added and mixed on a mechanical shaker for 10 min. The tubes were centrifuged at 4000rpm for 5min and 2.0 ml of the supernatant was taken.1.0 ml of DDC solution was added to all the tubes and shaken well. The colour intensity was read immediately at 430nm in a Shimadzu-UV spectrophotometer. Specific activity was expressed as the number of µM fatty acid liberated per mg protein.

3.12.4 Analysis of fatty acids (FAME)

Fatty acids were analyzed according to the method of AOAC, Gas chromatography method (1975). Lipid content of the tissues was estimated by the method of Folch *et al.,* (1957). Methyl esters of fatty acids from animal and vegetable origin having 8-24 atoms are separated and detected by gas chromatography. Method is not applicable to epoxy, oxidised, or polymerized fatty acids (Metcalfe *et al.,* 1966).

Reagents

1. Boron trifluoride reagent.
2. Methanolic sodium hydroxide solution.
3. Petroleum ether.
4. Sodium sulphate.

Procedure

Sample (lipid of known weight) was added to flask followed by methanolic NaOH and boiling chip. Condenser was attached and refluxed until fat globules disappear (usually 5-10 min). BF_3 solution was added from bulb or automatic pipette through condenser and boiling was continued for 2 min. Heat was removed, followed by condenser, and 15ml of saturated NaCl solution was added. Flask was stoppered and vigorously shook for 15s while solution was still tepid. Aqueous phase was transferred to 250ml separator. Extracted with two 30ml portions of petroleum ether (b.p 60-80^{o}C). Combined extracts were washed with 20ml portions of H_2O, dried over anhydrous Na_2SO_4, filtered and solvent was evaporated under stream of nitrogen on steam bath.

Methyl esters of the fatty acid thus obtained were separated by gas liquid chromatography (Varian CP 3800. U.S.A) equipped with a capillary column (30m long and 0.54mm diameter)

and a flame ionization detector in the presence of hydrogen and air. The carrier gas was nitrogen and the flow rate was 4ml/min the chromatograph temperature started at150^{0}C and was increased 4 ^{0}C/min until a temperature of 250 ^{0}C was obtained. Fatty acids separated were identified by the comparison of retention times with those obtained by the separation of a mixture of standard fatty acids. Measurement of peak areas and data processing were carried out by electronic integrator. Individual fatty acids were expressed as a percentage of total fatty acids.

3.13 Lipid peroxidation and tissue antioxidant status

3.13.1 Estimation of tissue lipid peroxides (LPO)

Serum lipid peroxide content was estimated by the method of Ohkawa *et al*., (1979).

Reagents

1. Sulphuric acid : 0.085 N
2. Phosphotungstic acid : 10%
3. TBA reagent : Mixture of equal volumes of 0.67 % TBA aqueous solution and glacial acetic acid.
4. n-Butanol

Procedure

0.1 ml of serum was mixed with 4.0 ml of 0.085N sulphuric acid and shake gently. To this 0.5 ml of phosphotungtic acid was added and stirred well. The contents were centrifuged for 10 min. The supernatant was discarded and the pellet was suspended in 2 ml 0.085 N sulphuric acid and 0.3 ml of 10% phosphotungtic acid. The mixture was centrifuged for 10 min. The pellet obtained was suspended in 4.0 ml of distilled water and 1.0ml of TBA reagent. The tubes were kept in a boiling water bath for one hour. After cooling 5.0 ml of n-butanol was added to each tube and the color obtained in butanol phase was read at 532nm in a Schimadzu-UVspectrophotometer. The serum lipid peroxide content was expressed as nmoles of malondialdehyde/ml.

3.13.2 Determination of total reduced glutathione (GSH)

The total reduced glutathione was determined by the method of Ellman (1959). The method is based on the reaction of reduced Glutathione with 5, 5'-dithiosbis (2-nitrobenzoic acid) (DTNB) to give a compound that has absorbance at 412 nm.

Reagents

1. DTNB: 0.6mM in 0.2M Phosphate buffer pH 8.0
2. Phosphate buffer: 0.2M, pH 8.0.
3. Trichloroacetic acid: 5%
4. Standard: 61.4mg of reduced glutathione was dissolved in 100 ml .02M EDTA 0.1 ml of this is made up to 10 ml with 0.02M EDTA. Working standard: 2.0 ml of the above was made up to 10 ml.

Procedure

0.5 ml of liver homogenates was precipitated with 5% of TCA. The contents were mixed well for complete precipitation of proteins and centrifuged at 4000rpm for 5min. To an aliquot of clear supernatant, 2.0 ml of DTNB reagent and 0.2M Phosphate buffer were added to make a final volume of 4.0 ml. The absorbance was read at 412nm against a blank containing TCA instead of sample, series of standards treated in a similar way were also run to determine the reduced glutathione content. The amount of glutathione was expressed as µ moles/g wet tissue.

3.13.3 Estimation of glutathione peroxidase (EC 1.11.1.9)

The method of Paglia & Valentine (1967) was adopted for assay of glutathione peroxidase.

Reagents

1. 0.4 M Phosphate buffer, pH 7.
2. 0.4M Tris buffer, pH 8.9
3. 0.4mM EDTA
4. 2mM GSH

5. 10mM NaN3
6. 10% TCA
7. DTNB: 99mg in 25 ml of methanol.
8. H_2O_2: 1mM was prepared freshly from commercial 30%solution.
9. GSH standard: 61.4mg GSH was dissolved in 100 ml distilled water.1.0 ml of this solution was made up to 10 ml with distilled water. Working standard: 2 ml of the stock was made up to 10 ml with distilled water.

Procedure

0.2 ml of tissue homogenate was added to a mixture containing 0.2 ml of buffer, 0.2 ml of EDTA, 0.1 ml of sodium azide, mixed well and added 0.1 ml reduced glutathione and 0.1 ml of hydrogen peroxide. Incubated in a water bath at 37^{o}C for 10min. At the end of incubation period, 0.5ml of 10%TCA was added and centrifuged at 10000rpm for 5 min. 1.0 ml of the supernatant was taken into a separate test tube and added 2.0 ml Tris buffer and 50 µl DTNB. Immediately read the OD at 412nm. The enzyme activity was expressed as n moles of glutathione oxidized per min per mg protein.

3.13.4 Assay of glutathione-s-transferase (EC 2.5.1.18)

Glutathione-S-tranferase activity was determined by the method of Habig *et al.*, (1974).

Reagents

1. Phosphate buffer :0. 3 M, pH 6.5
2. 1-chloro-2, 4-dinitrobenzene (CDNB): 30mM
3. Reduced Glutathione (GSH): 30mM.

Procedure

The reaction mixture containing 1.0 ml of buffer, 0.1 ml of CDNB and 0.1 ml of tissue homogenate was made up to 2.5 ml with water. The reaction mixture was pre-incubated at 37oC for 5min. 0.1 ml of GSH was added and the change in the absorbance was measured at 340nm for 3 min at 30 sec intervals. The enzyme activity was expressed as µmoles of CDNB conjugate formed/min/mg protein.

3.13.5 Assay of catalase (EC 1.11.1.6)

Catalase was assayed according to the method of Takahara *et al.,* (1960).

Reagents

1. Phosphate buffer: 50mM, pH 7.0
2. Hydrogen peroxide: 30mM solution in the above buffer.

Procedure

To 2.0 ml of the phosphate buffer, 50 µl of the enzyme source was added and the reaction was started by the addition of 1.0 ml of H_2O_2 solution. The decrease in absorbance was measured at 240nm at 30 sec intervals for 2 min. The enzyme blank was run simultaneously with 1.0 ml of distilled water instead of hydrogen peroxide. The enzyme activity was expressed as n moles of H_2O_2decomposed per minute per mg protein.

3.13.6 Assay of superoxide dismutase (EC 1.15.1.1)

The superoxide dismutase was assayed according to the method of Misra and Fridovich (1972) based on the oxidation of epinephrine-adrenochrome transition by the enzyme.

Reagents

1. 0.1M Carbonate-bicarbonate buffer: pH 10.2 containing 57mg/dl EDTA.
2. Epinephrine: 3mM

Procedure

Taken 50 µl of sample into the cuvette and add 1.5 ml buffer and 0.5 ml epinephrine mixed well and immediately read the change in optical density at 480nm for 2min in a Shimadzu-UV spectrophotometer. One unit of SOD activity was the amount of protein required to give 50% inhibition of epinephrine auto oxidation.

3.14 Membrane-bound ATPases

3.14.1 Assay of Na+/K+ -dependent ATPase

Na+/K+ dependent ATPase activity was measured from the amount of Pi released according to the method of Bonting (1970).

Reagents

1. 184mM Tris buffer, pH 7.
2. 50mM Magnesium Sulphate
3. 50mM Potassium Chloride
4. 600mM Sodium Chloride
5. 1.0mM EDTA
6. 40mM ATP
7. 10% TCA

Procedure

One ml of Tris-buffer and 0.2 ml each of the above assay reagents were mixed together. So the assay medium, in the final volume of 2.0 ml contained, 92mM Tris-buffer, 5mM magnesium sulphate, 60mM sodium chloride, 5mM potassium chloride, 0.1mM EDTA and 4.0mM ATP. After 10 min equilibration at 37^{o}C in an incubator, reaction was started by the addition of 0.2 ml of the enzyme solution. The assay medium was incubated for 30 min and at the end of the incubation period. The reaction was stopped by the addition of 2 volumes of ice cold 10% TCA. The phosphorus (Pi) liberated was estimated by the method of Fiske and Subbarow (1925). The enzyme activity was expressed as µmoles of Pi liberated per min per mg protein.

3.14.2 Assay of Ca 2+-dependent ATPase

Ca 2+ Dependent ATPase was assayed by the method of Hjerten and Pan (1983).

Reagents

1. 0.125M, pH 8.0 Tris-HCl buffer.
2. 0.05M Calcium chloride
3. 0.01M ATP
4. 10%TCA

Procedure

Tris-HCl buffer 0.1 ml, Calcium 0.1 ml, ATP solution 0.1 ml and distilled water 0.1 ml were taken in test tubes. 0.1 ml of enzyme preparation was added and the tubes were incubated at 37^{o}C for 15 min. The reaction was arrested by the addition of 10% TCA to the incubation mixture.0.1 ml enzyme source was added to the control tubes. The contents were centrifuged at 4000rpm for 5 min. The supernatant was used for the estimation of inorganic phosphorous.

The enzyme activity was expressed as µmoles of Pi liberated per mg protein per min.

3.14.3 Estimation of inorganic phosphorus

Inorganic Phosphorus was estimated by the method of Fiske and Subbarow (1925). The method is based on the formation of phoshomolybdic acid by the reaction between a phosphate and molybdic acid and its subsequent reduction to a dark blue phosphomolybdic acid, the intensity of which is proportional to the phosphate ion concentrate on.

Reagents

1. Ammonium molybdate reagent: 2.5g of ammonium molybdate was dissolved in 100 ml of 3N sulphuric acid.
2. Amino naphthol sulphonic acid (ANSA): .5g of ANSA was dissolved in 195 ml of 15% sodium metabisulphite and 5.0 ml of 20% sodium sulphate was added for complete solubilization. The solution was filtered and stored in a brown bottle.
3. Standard Phosphorus: 35.1mg of potassium dihydrogen phosphate was accurately weighed, dissolved and made up to 100 ml with distilled water.

Procedure

To suitable aliquots of the supernatant, 1.0 ml of ammonium molybdate reagent was added.0.4 ml of ANSA was added after 10 min incubation at room temperature, standards and blank were also treated in the above manner. The blue colour developed was read after 20 min at 640nm in a Shimadzu-UV Spectrophotometer. The values were expressed as µg per mg protein.

3.15 Preparation of mitochondrial-rich fraction from liver.

Reagents

1. Tris HCl: 50mM, pH 7.4 containing 0.25 M Sucrose and 1mM EDTA.

Procedure

Immediately after sacrifice, the liver was removed and all the blood vessels and connective tissues were trimmed off. Wash the tissue free of blood in ice-cold sucrose, lightly blot and place in a tared beaker to weigh. Cut the liver in to small fragments and homogenize in buffer containing 0.25 M Sucrose and 1mM EDTA. Centrifuge the suspension in a refrigerated centrifuge (Susin, 2000).

The homogenate was centrifuged at 1000g for 10 min; the supernatant was transferred in to test tubes. The pellet was dissolved in sucrose buffer and centrifuged for 10 min at 1000g. The supernatants were pooled and centrifuged for 10 min at 10000g, the pellet collected represents mitochondrial fraction. Each fraction was resuspended in sucrose and the washings combined with the supernatants. This has the advantage of producing purer fractions. Carefully the mitochondrial pellet was resuspended in about 2ml of sucrose and used as the enzyme source and store on ice until required.

3.15.1 Mitochondrial and respiratory marker enzymes

3.15.1.1 Assay of Isocitrate dehydrogenase (EC 1.1.1.42)

The enzyme activity was assayed according to the method of Bell & Baron (1960)

Reagents

1. Tris-HCl buffer : 0. 1 M, pH 7.5
2. Substrate : 0. 1M trisodium DL-Isocitrate in 0.9% saline
3. Manganous chloride : 0. 015 M in 0.9% saline
4. NADP : 0. 001 M in 0.9% saline
5. EDTA : 5%
6. Sodium hydroxide : 0. 4 N
7. 2,4 dinitrophenyl hydrazine (DNPH) : 0. 001 M in 1N HCl
8. Standard Solution : 15 mg of α-ketoglutarate in 50ml of buffer**.**

Procedure

Buffer, 0.4ml was taken in a test tube and 0.2ml of substrate, 0.3ml of manganous chloride and 0.2ml of the mitochondrial suspension were added. A control tube was also prepared simultaneously, 0.2ml of co-enzyme solution was added to the test tube and 0.2ml of saline was added to the control tubes. After mixing well, both the tubes were incubated for 60 min, 1.0 ml of DNPH was added to both the tubes, followed by 0.5 ml of EDTA. The tubes were kept at room temperature for 20 min and 10 ml of 0.4 N NaOH was added to the tubes. A blank was run simultaneously. The colour intensity was measured at 390 nm in a Shimadzu- UV spectrometer. A standard curve was prepared using α-ketoglutarate.

The activity of isocitrate dehydrogenase is expressed as μ moles of α-ketoglutarate liberated per mg protein per hour.

3.15.1.2 Assay of Succinate dehydrogenase (EC 1.3.99.1)

This enzyme activity was estimated accordingly to the method of Slater and Bonner (1952). The rate of reduction of potassium ferricyanide was measured in the presence of sufficient potassium cyanide to inhibit cytochrome oxidase by following the rate of decrease in the optical density at 420 nm.

Reagents

1. Phosphate buffer : 0.3 M pH 7.6
2. Sodium salt of (EDTA : 0.03 M solution.
3. Potassium cyanide : 0.03 M solution.
4. Sodium Succinate : 0.4 m solution.
5. Bovine serum albumin : 3% solution
6. Potassium ferricyanide : 0.075 M solution

Procedure

In a spectrophotometric cuvette, 1.0 ml of phosphate buffer, 0.1 ml of EDTA, 0.1 ml of bovine serum albumin, 0.3 ml of sodium succinate, 0.2 ml of potassium ferricyanide and 0.1 ml of potassium cyanide were added and the total volume was made up to 2.8 ml with double distilled water. The reaction was started by the addition of 0.2 ml of mitochondrial suspension. Changes in optical density at 420 nm were recorded in a Shimadzu- UV

spectrophotometer at an interval of 15 s for 5 min. The activity of succinate dehydrogenase is expressed as per n moles of succinate oxidized/ minute /mg protein.

3.15.1.3 Assay of Malate dehydrogenase (EC 1.1.37)

This enzyme activity was assayed by the method of Mehler et *al* 1948. The activity determination is based on the measurement of the rate of oxidation of NADH in the presence of the enzyme and excess of oxaloacetate.

Reagents

1. Tris – HCl : 0.25M, pH 7.4
2. NADH : 0.015 M
3. Oxaloacetate : 0.0076 M, pH 7.4

Procedure

To 0.3 ml of buffer 0.1 ml of NADH and 0.1 ml of oxaloacetate were added and the total volume was made to 2.9 ml with water. The reaction was started by adding 0.1 ml of mitochondrial suspension. The change in optical density was measured at 340 nm in a Shimadzu- UV spectrophotometer at intervals of 15 s for 5 min. The activity of malate dehydrogenase was expressed as n moles of NADH oxidized per minute per mg of protein.

3.15.1.4 Assay of NADH dehydrogenase (EC 1.6.99.3)

The activity of NADH dehydrogenase was assayed according to the method of Minakami *et al.,* 1962.

Reagents

1. Phosphate buffer : 0.1 M, pH 7.4.
2. NADH : 0.1% solution
3. Potassium ferricyanide : 0. 03 M solution

Procedure

The reaction mixture contained 1.0ml of phosphate buffer, 0.1 ml of potassium ferricyanide, 0.1 ml of NADH and 1.6 ml of distilled water in a total volume of 3.0 ml. The

temperature was brought to 30^0 C and NADH was added just before the addition of the sample. A suitable aliquot of mitochondrial solution was added and change in absorbance was measured at 420 nm as function of time 3 min at intervals of 15 seconds in a Shimadzu-UV spectrophotometer. A control containing all the reagents except NADH was also treated similarly. The activity of NADH-dehydrogenase is expressed as per n moles of NADH oxidized per minute per mg of protein.

3.15.2 Mitochondrial Lipid peroxidation and antioxidant status.

3.15.2.1 Determination of lipid peroxides

The heart mitochondrial lipid peroxide content was determined by the thiobarbituric acid reaction as described by Ohkawa *et al.,* (1979)

3.15.2.2 Assay of superoxide dismutase

The mitochondrial superoxide dismutase activity was assayed by the method of Misra & Fridovich (1972). One unit of SOD activity is the amount of protein required to give 50% inhibition of epinephrine autoxidation.

3.15.2.3Assay of catalase

The mitochondrial catalase activity was assayed by the method of Takahara (1960).The enzyme activity was expressed as µ moles of H_2O_2 consumed/min/mg of protein.

3.15.2.4 Determination of mitochondrial total reduced glutathione

The total reduced glutathione in heart mitochondria was determined according to the method of Ellamn (1959). The amount of glutathione is expressed as n mol/g tissue.

3.15.2.5 Assay of glutathione peroxidase.

The mitochondrial glutathione peroxidase activity was assayed by the method of Habig *et al.,* (1974). The enzyme activity is expressed as n mol of glutathione oxidized/ min/mg protein.

3.15.2.6 Assay of glutathione-S-transferase

The mitochondrial glutathione-S-transferase activity was assayed by the method of Pagila & Valentine (1967). The enzyme activity is expressed as n mol of CDNB conjugate formed/min/protein.

3.15.3 Membrane bound ATP-ases

3.15.3.1 Assay of Ca^{2+} dependent ATPase

The mitochondrial Ca^{2+} dependent ATPase activity was assayed by the method of Hjerten and Pan (1983). The enzyme activity is expressed as µ moles of Pi liberated/ min/mg of protein.

3.15.3.2 Assay of Total ATPase

The mitochondrial total ATPase activity was assayed by the method of Hokins *et al.*, (1973) which was modified from the method of Evans (1993). The enzyme activity is expressed as µ mol of Pi liberated/ min/mg of protein.

3.16 Statistical analysis

Results were expressed as means ± SD, and One-way ANOVA and Student's *t*-test were used to assess statistical significance. All data were analyzed with the aid of statistical package program SPSS 10.0 for Windows.

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 CCl_4 (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.,* 2 001).

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 (Tasci *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 cycles 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 (F**ig 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 (Doğ ru-Abbasoğ lu, 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 hypoglyceamia 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 phospahatase 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-l, 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 demonstated 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) a**nd 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 hepatoxicity 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-toxiicty causes accumulation of fat in liver and increases lipid levels in serum.

Administration with taurine has caused the blood levels of total cholesterol andLDL 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 7α–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 blood stream 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 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 phopholipase 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 apotosis (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 to macromolecular and membrane damage and disruption of signaling pathways are known to to stimulate hepatocyte injury 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. Mordern day diet has a severly 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 celluar 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_2O_2 and water. CAT is a hemeprotein, localized in the peroxisomes that catalyses the decomposition of H_2O_2 to water and oxygen and thus protecting the cell from oxidative damage by H_2O_2 and $OH^{\cdot}$. 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 2nd 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 lipd 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 Kuppfer 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 CCl_4-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 GSSH 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 H_2O_2 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_2O_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 ($OH^\bullet$) 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

Na^+, K^+-ATPase is a plasma membrane-bound enzyme that provides the necessary electrochemical gradients of Na^+ and K^+ to maintain the cell volume and thus it plays a crucial role in homeostasis. It functions by exporting intracellular Na^+ and importing extracellular 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, Na^+, K^+-ATPase and 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^{2+}ATPase and Mg^{2+}ATPase are membrane bound enzymes that catalyses the active transport of Ca^{2+} and Mg^{2+} across the cell membrane to maintain low intracellular Ca^{2+} and Mg^{2+} content. The intracellular concentration of calcium regulates the activity of the Mg^{2+}- and Na^{+}, K^{+}-ATPases. Disturbances in the function of Ca^{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-excitable tissues. Lipid peroxidation induced by free radicals has been shown to inactivate Na^{+}, K^{+}-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 activites of the memebrane 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^{2+}-, Mg^{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^{+}, K^{+}-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^{+}, K^{+}-ATPase to function properly (Huxtable, 1992 & Timbrell *et al.,* l 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^+/K^+ATPase activity by taurine could produce a decrease in uptake of Ca^{2+} due to the decreased activity of Na^+/Ca^{2+} exchanger. Sebring and Huxtable, 1985 showed that taurine stimulates the pumping rate of Ca^{2+}-activated ATPase pump possibly by increasing the turnover rate of the pump secondary to a membrane modification. The membrane activities of Na+, K(+)-ATPase, Mg2+, Ca(2+)-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 $Na^+ K^+$ 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^+, K^+-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^+, K^+-ATPase function through membrane damage. Disability of the pump results in failure of the cell to actively drive out Na+ causing its accumulation in the liver cell. Also in an injured cell, the rise in intracellular sodium concentration will lead to enhanced Ca^{2+}influx and increased intracellular Ca^{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 Na^+/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 dehydragenase, 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,

1969). 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 cytchrome-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 hepatotoxicities 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 mitochiondria are crucial for cells to stay healthy. In several forms of liver injury the mitochondrial membrane potential and the sensitivity to mitochiondrial 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*

al., 2000; Sener *et al.*, 2005; Schaffer *et al.*, 2003; Parvez *et al.*, 2008) 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 anerobic 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.

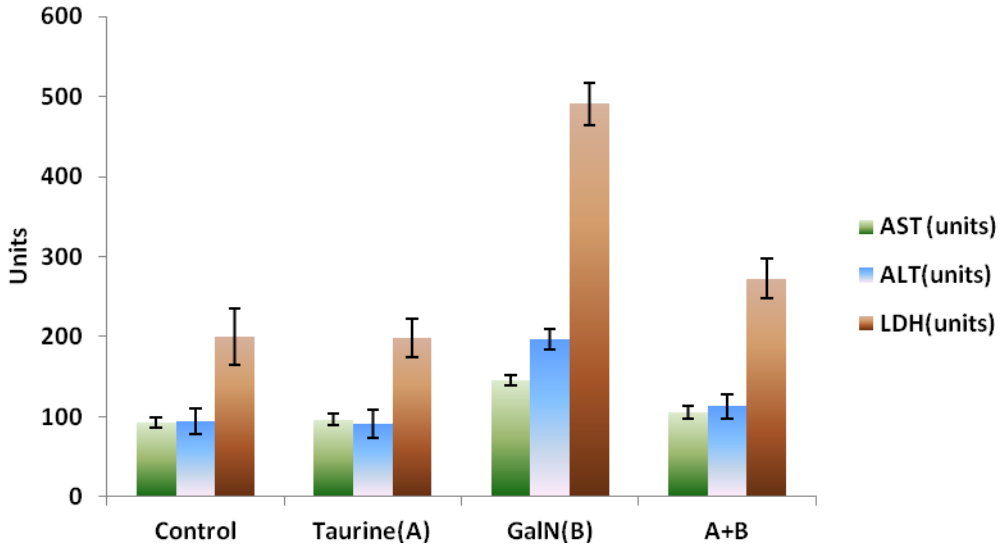


Fig 4.1.1 Level of aspartate amino transferase (AST), alanine amino transferase (ALT) and lactate dehydrogenase (LDH) in plasma of control and experimental rats

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; 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

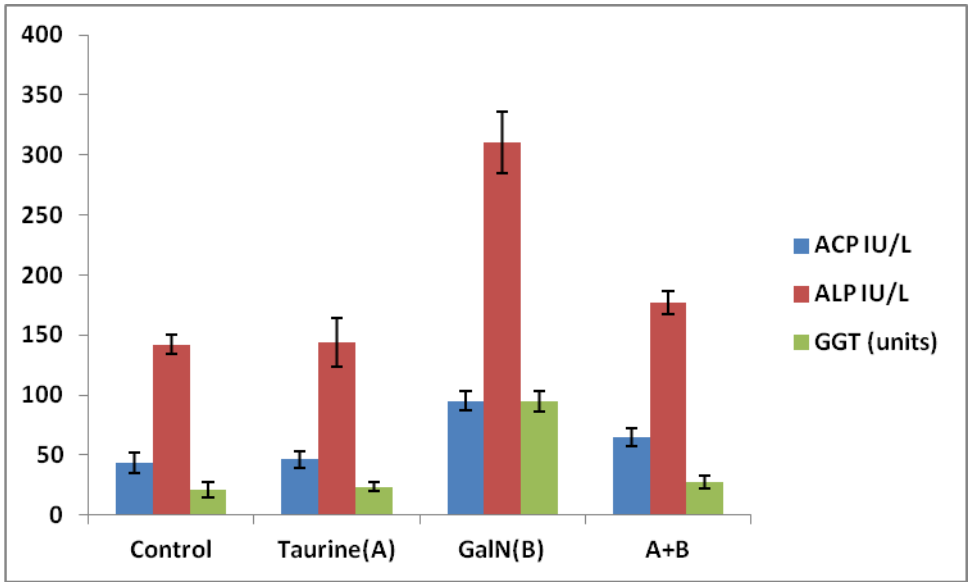


Fig 4.1.2 Levels of alkaline phosphotase, acid phosphatase and γ-glutamyl transferase in plasma of control and experimental rats

For **γ-glutamyl transferase one unit is equivalent to µg 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.

[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

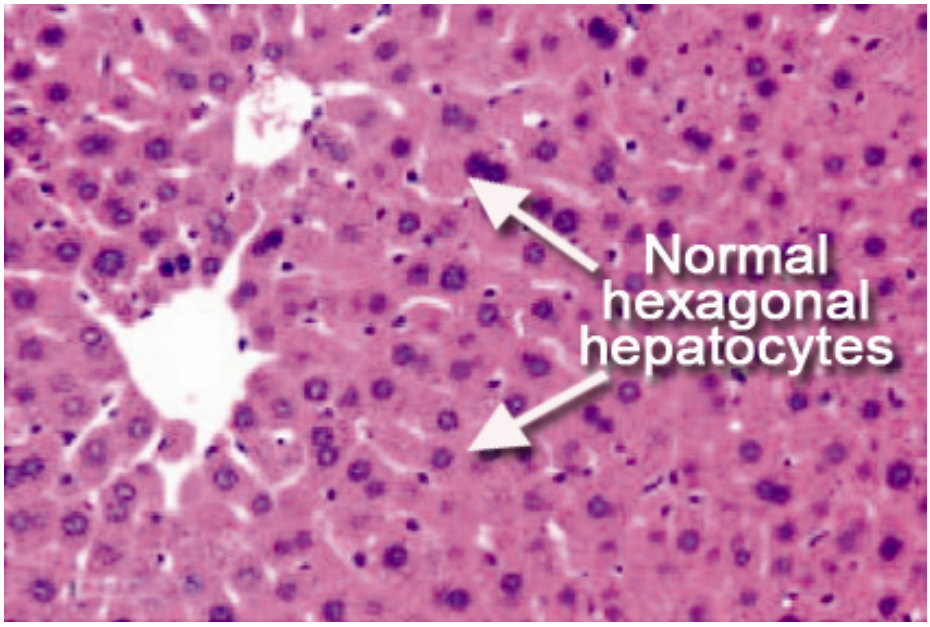


Plate 4.2.1 The architecture of normal liver tissue in control rats. Stained with haematoxylin-eosin; 20X magnification.

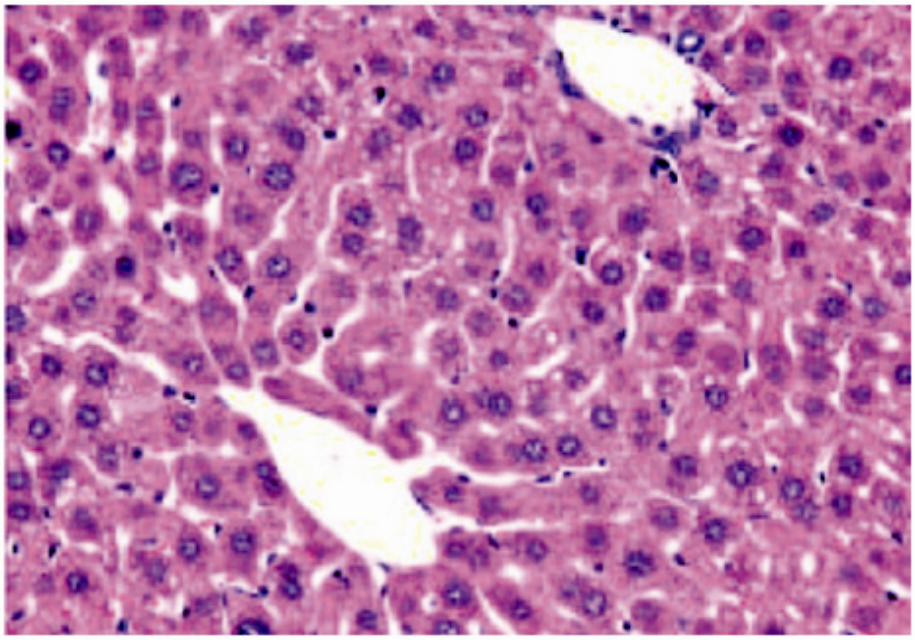


Plate 4.2.2 **The liver tissue in rats administered with taurine indicating no significant changes in architecture in comparison to the normal condition**. **Stained with haematoxylin-eosin; 20X magnification.**

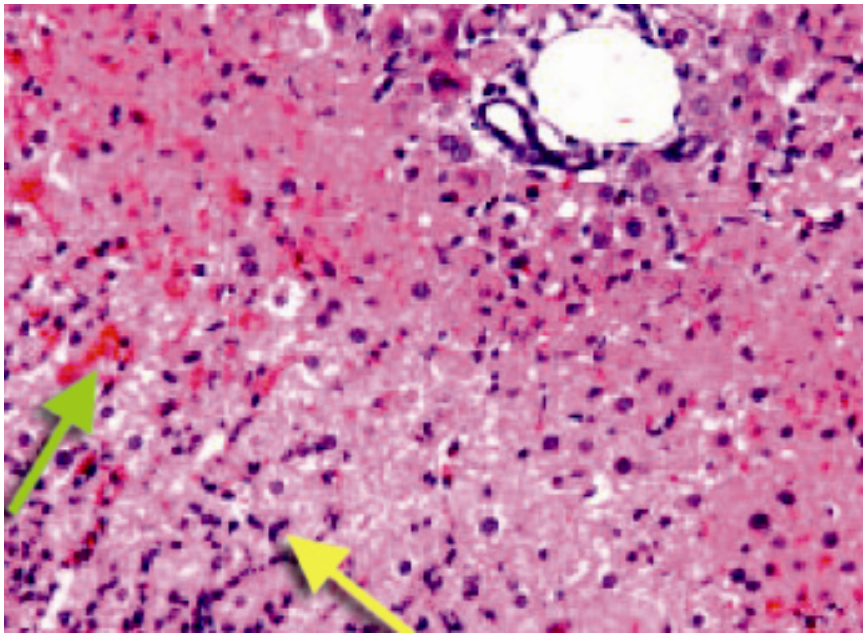


Plate 4.2.3 The architecture of liver tissue in D-galactosamine-administered rats showing necrosis (green arrow) with inflammatory cells (yellow arrow). Stained with haematoxylin-eosin; 20X magnification.

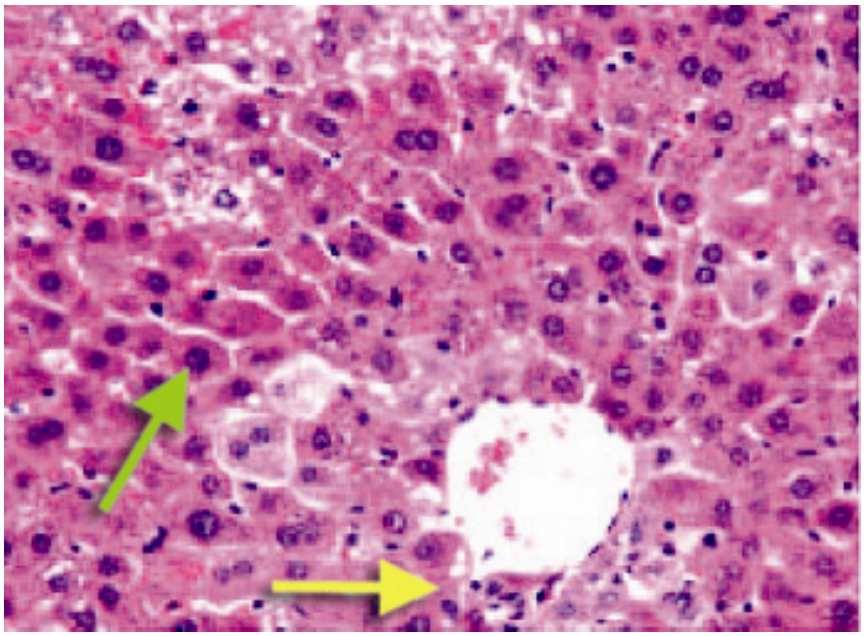


Plate 4.2.4 The architecture of liver tissues in rats treated with taurine and D-galactosamine, showing marked reduction in necrosis (green arrow) and inflammatory cells (yellow arrow). Stained with haematoxylin-eosin; 20X magnification.

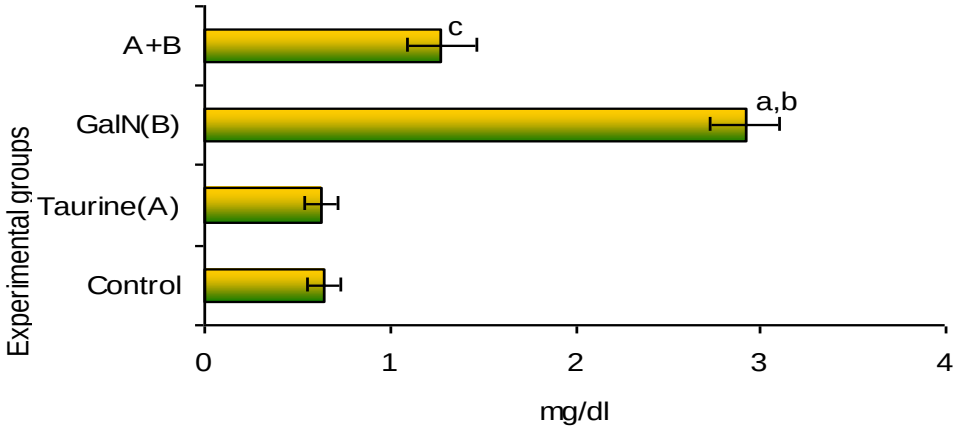


Fig 4.3.1 Levels of bilirubin in serum of control and experimental groups of rats

(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; 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

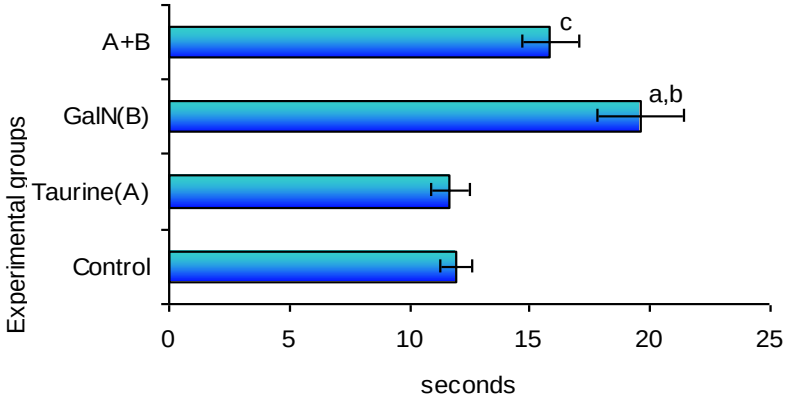


Fig 4.3.2 Levels of prothrombin time of control and experimental groups of rats

(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; 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

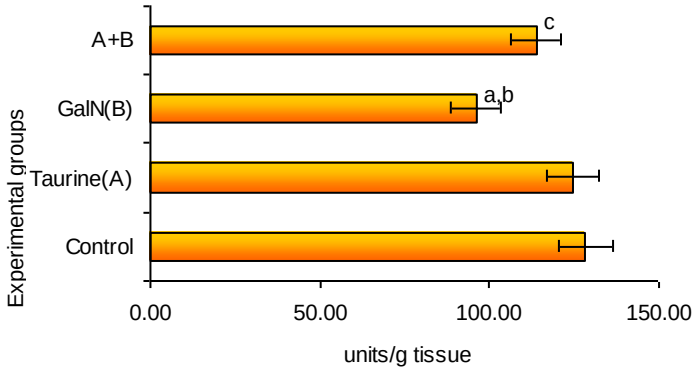


Fig 4.4.2 Levels of arginase in liver of control and experimental groups of rats

(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; 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

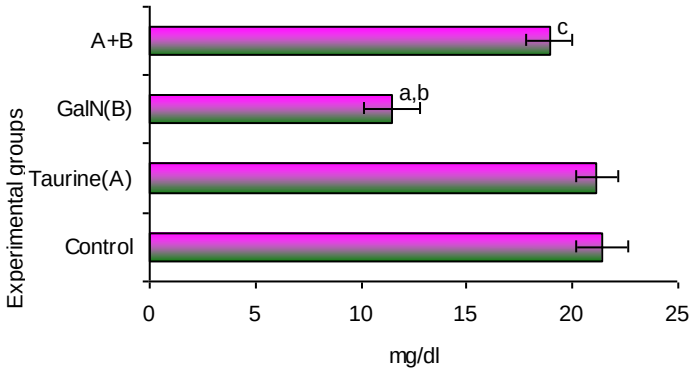


Fig 4.4.3 Levels of urea in serum of control and experimental groups of rats

(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; 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

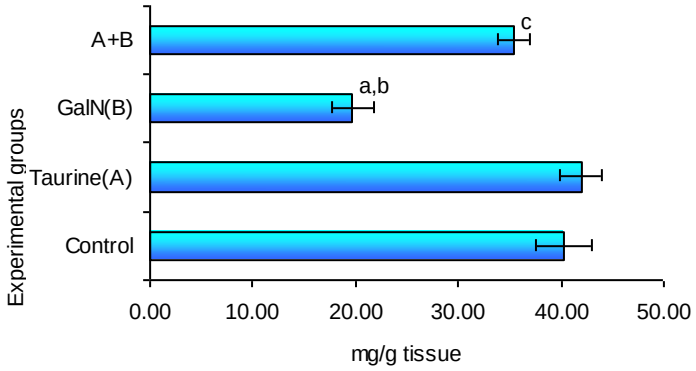


Fig 4.5.1.1 Levels of glycogen in liver of control and experimental groups of rats

(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

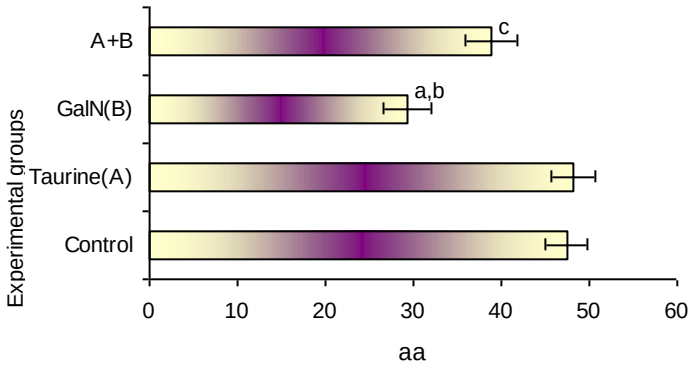


Fig 4.5.1.2 Activity of glycogen phosphorylase in liver of control and experimental groups of rats

(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

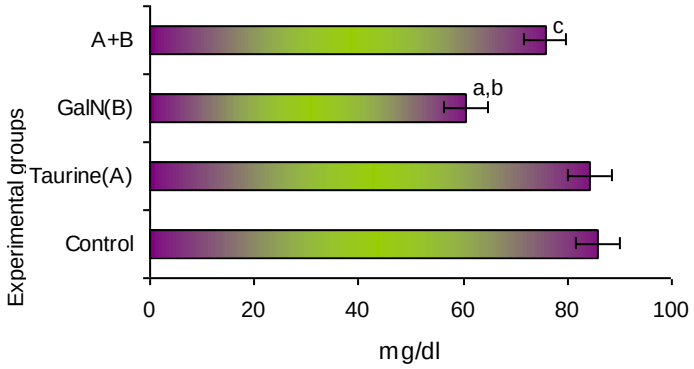


Fig 4.5.2.1 Levels of glucose in blood of control and experimental groups of rats

(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

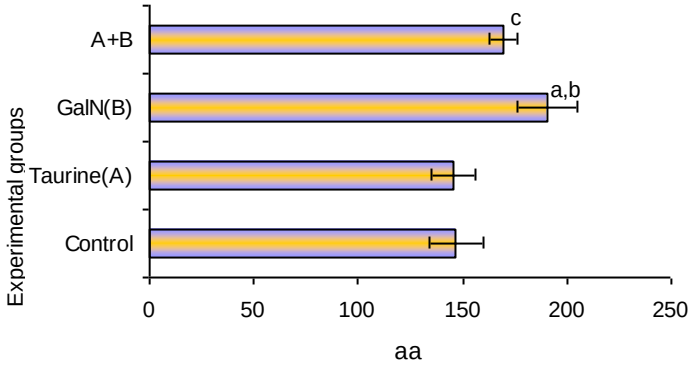


Fig 4.5.2.2 Activity of hexokinase in liver of control and experimental groups of rats

(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

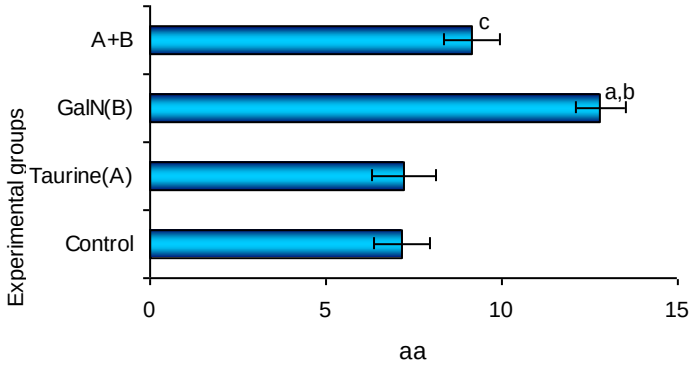


Fig 4.5.2.3 Activity of glucose-6-phosphate dehydrogenase in liver of control and experimental rats

(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; 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 rat

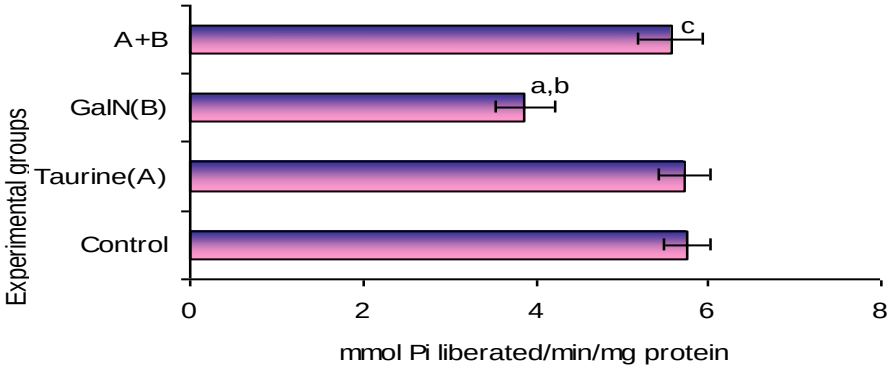


Fig 4.5.2.4 Activity of glucose-6-phosphatase in liver of control and experimental groups of rats

(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; 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

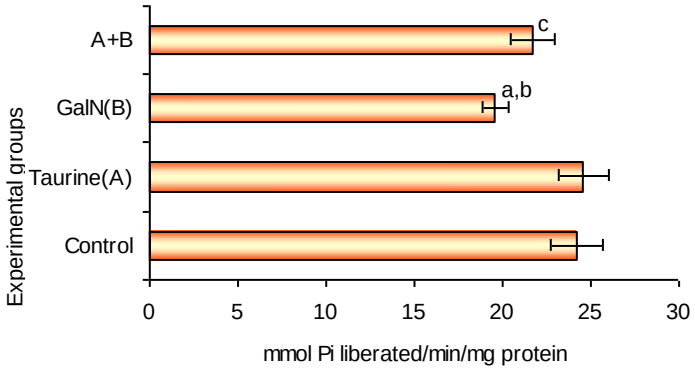


Fig 4.5.2.5 Activity of fructose 1,6 bis phosphatase in liver of control and experimental rats

(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

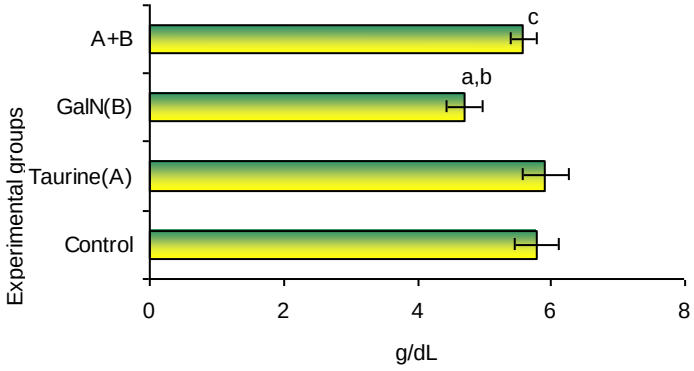


Fig 4.6.1 Level of protein in plasma of control and experimental groups of rats

(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

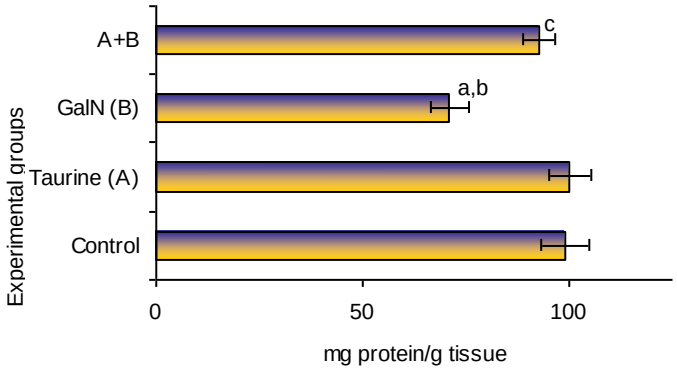


Fig 4.6.2 Level of protein in liver tissue of control and experimental groups of rats

(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; Oneway ANOVA; SPSS vers ion 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

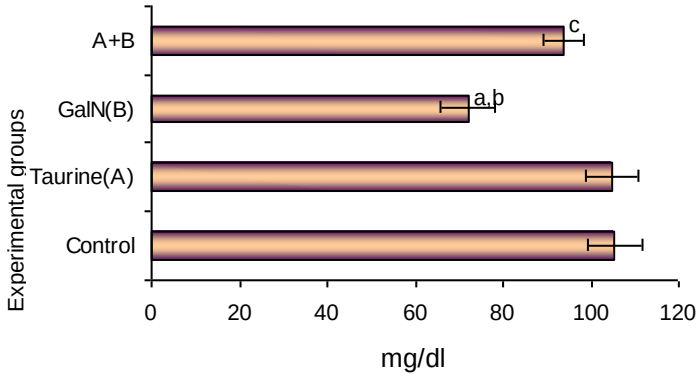


Fig 4.6.3 Levels of hexose in plasma of control and experimental groups of rats

(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; 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

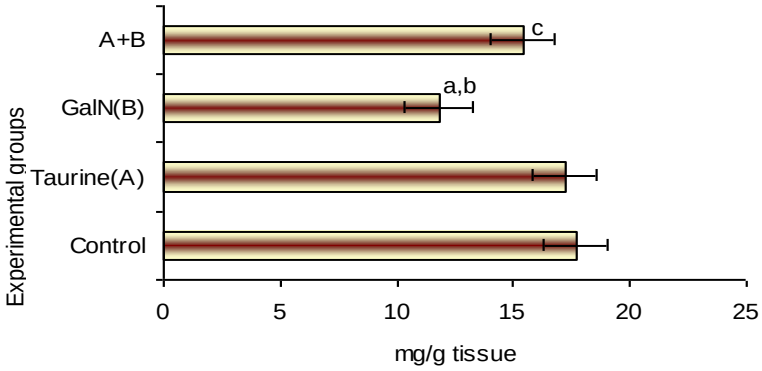


Fig 4.6.4 Levels of hexose in liver tissue of control and experimental groups of rats

(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; 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

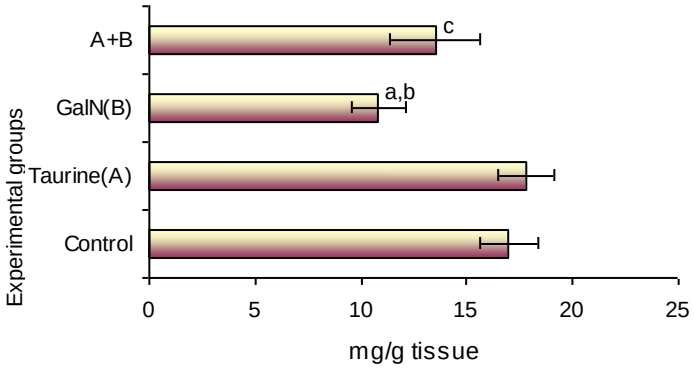


Fig 4.6.5 Levels of hexoseamine in liver tissue of control and experimental rats

(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; 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

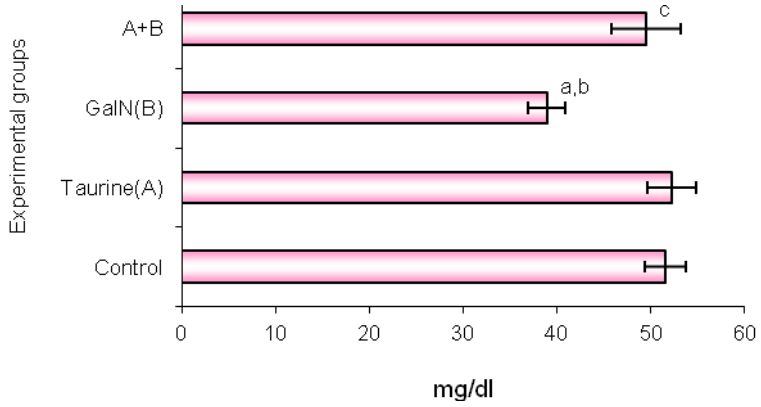


Fig 4.6.6 Levels of sialic acid in plasma of control and experimental group of rats

(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

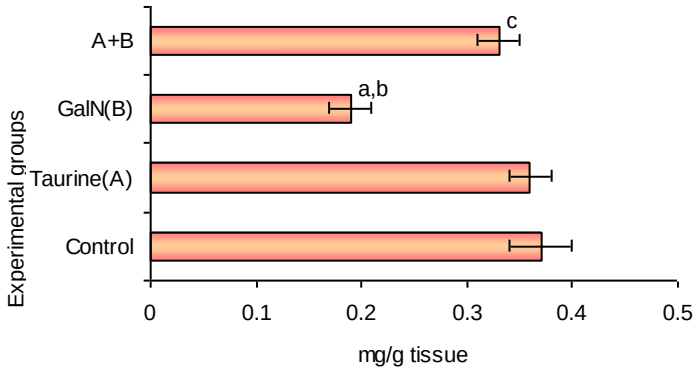


Fig 4.6.7 Levels of sialic acid in liver tissue of control and experimental group of rats

(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

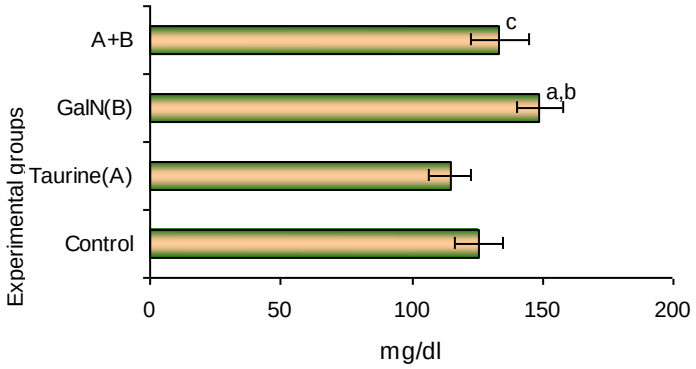


Fig 4.7.1.1 Level of total cholesterol in plasma of control and experimental groups of rats
(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 30 days

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

Results are mean ± SD for 6 animals; 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

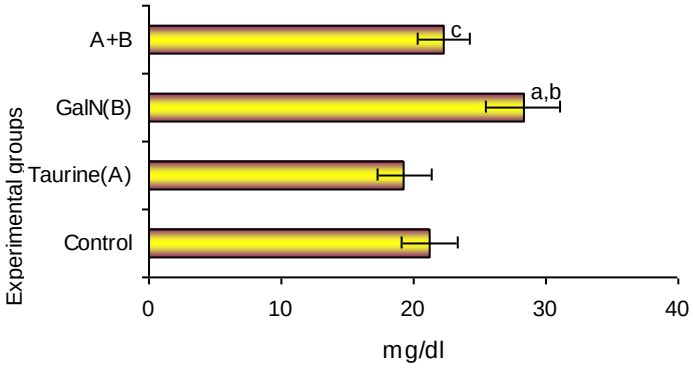


Fig 4.7.1.2 Level of VLDL-cholesterol in plasma of control and experimental rats

(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; 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

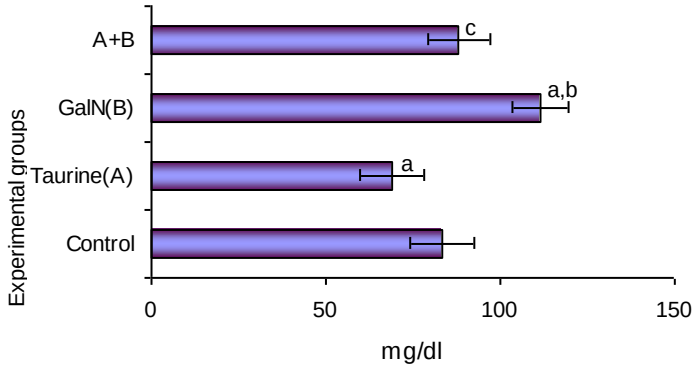


Fig 4.7.1.3 Level of LDL-cholesterol in plasma of control and experimental groups of rats

(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; 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

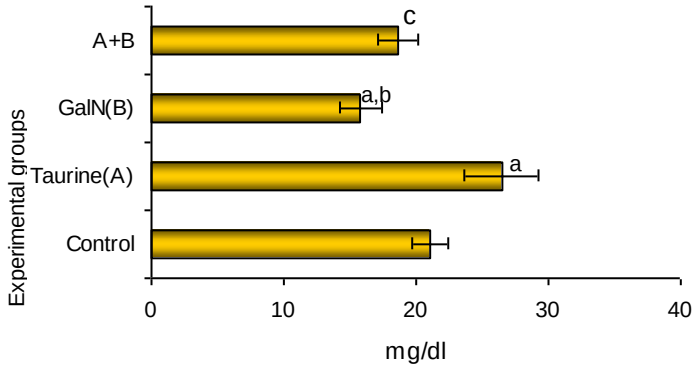


Fig 4.7.1.4 Level of HDL-cholesterol in plasma of control and experimental groups of rats

(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; 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

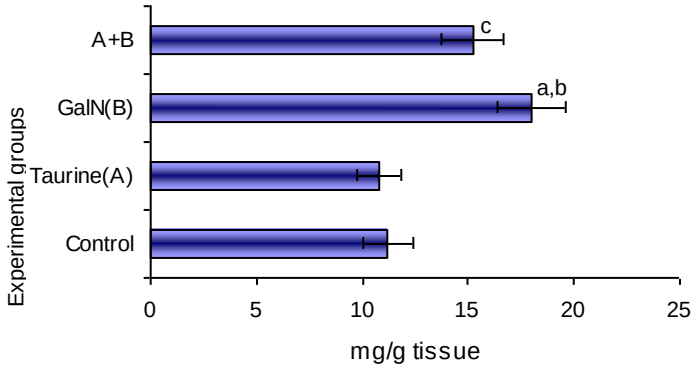


Fig 4.7.1.5 Level of total cholesterol in liver of control and experimental groups of rats

(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 rat

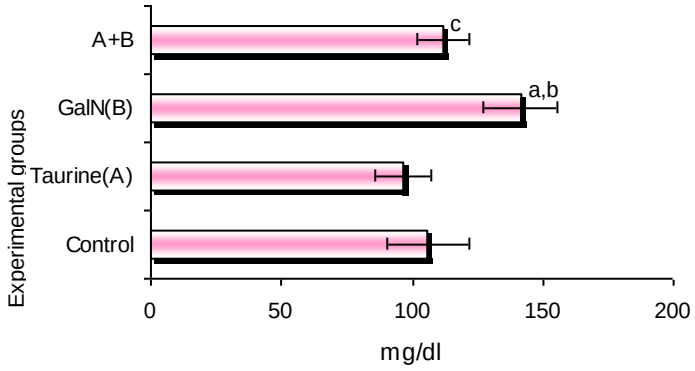


Fig 4.7.2.1 Level of triglycerides in plasma of control and experimental groups of rats

(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

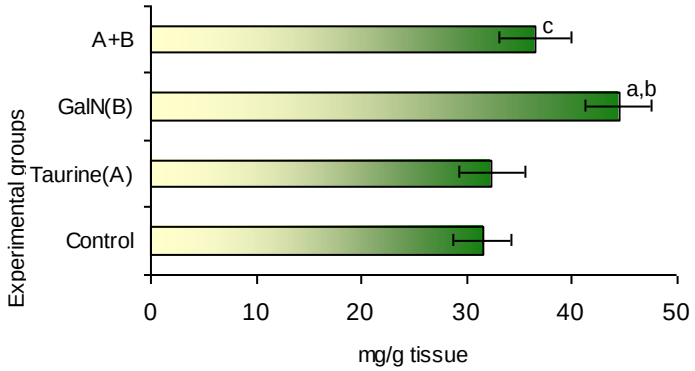


Fig 4.7.2.2 Level of triglycerides in liver tissue of control and experimental groups of rats

(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

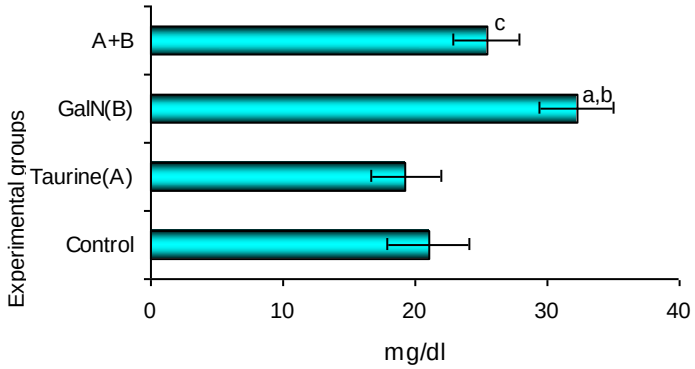


Fig 4.7.2.3 Level of free fatty acids in plasma of control and experimental groups of rats

(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

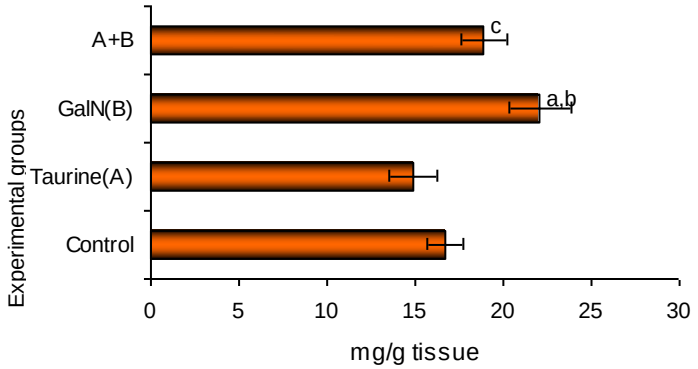


Fig 4.7.2.4 Level of free fatty acids in liver tissue of control and experimental groups of rats

(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.

[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

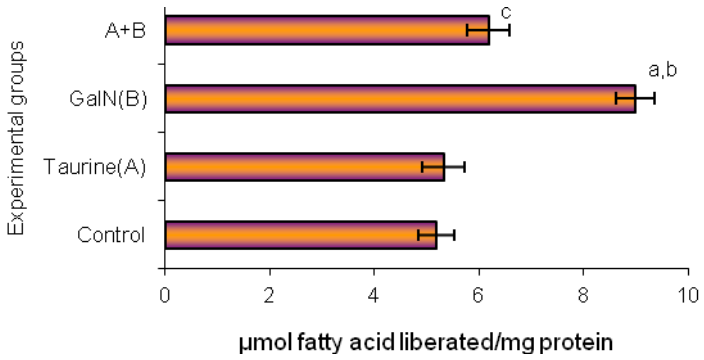


Fig 4.7.2.5 Activity of triglyceride lipase in liver of control and experimental groups of rats

(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.

[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

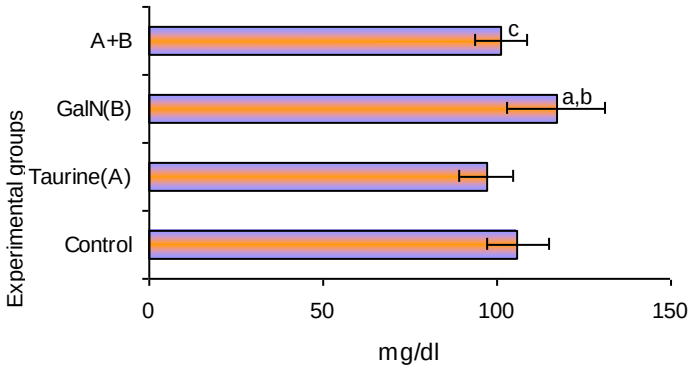


Fig 4.7.3.1 Level of phospholipids in plasma of control and experimental groups of rats

(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

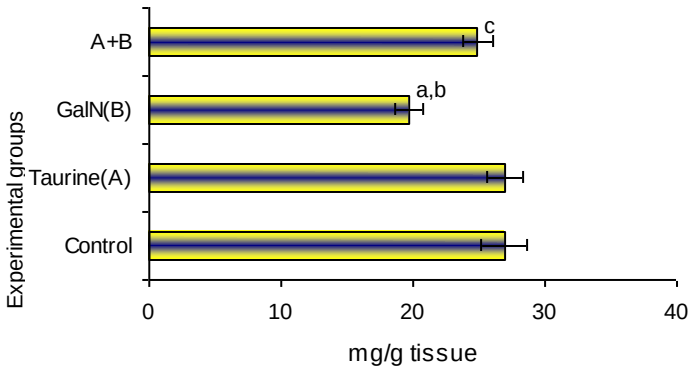


Fig 4.7.3.2 Level of phospholipids in liver tissue of control and experimental groups of rats

(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

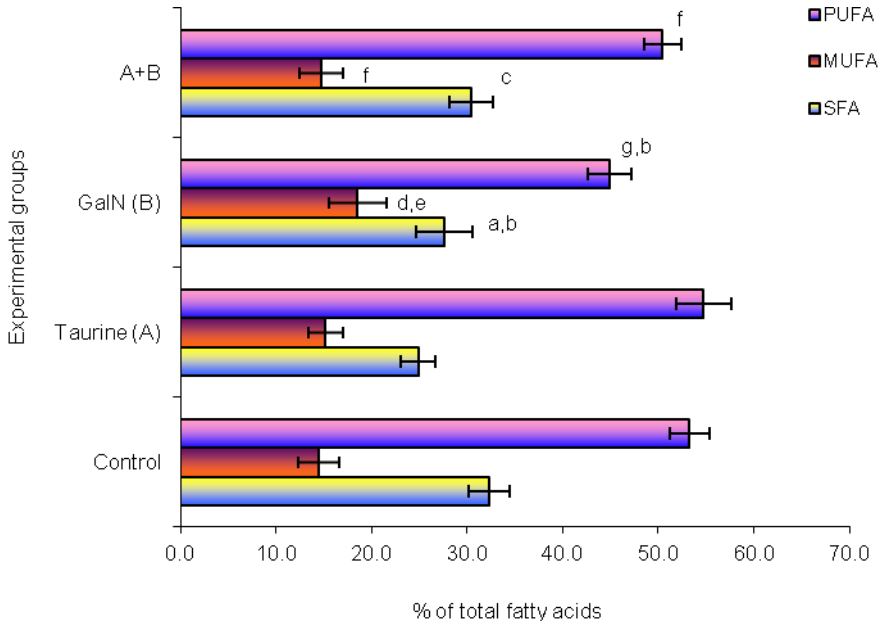


Fig 4.7.4.1 Levels of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in liver tissue of control and experimental groups of rats

(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; 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 galactosamine-induced hepatic failure rats

[d]$p<0.05$ significantly different compared with Group I control animals

[e]$p<0.05$ significantly different compared with Group II taurine-administered animals

[f]$p<0.05$ significantly different compared with Group III galactosamine-induced hepatic failure rats

[g]$p<0.01$ significantly different compared with Group I control animals

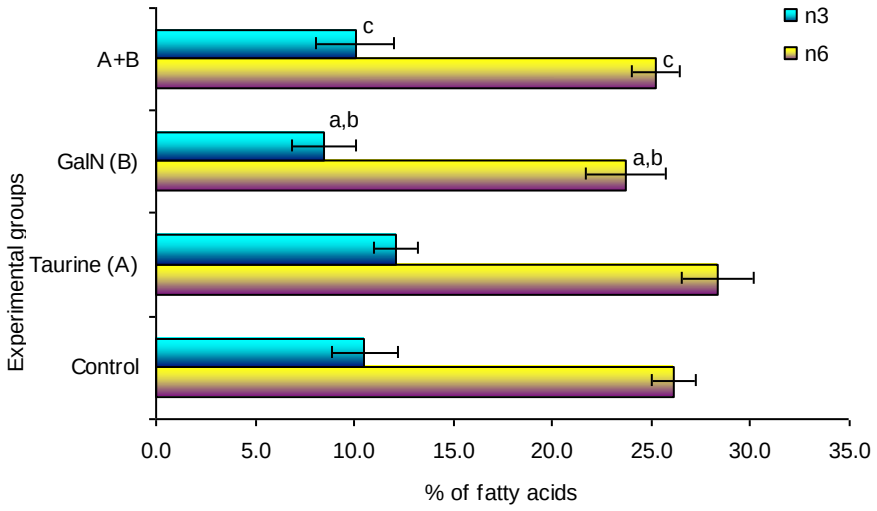


Fig 4.7.4.2 Levels of n6 and n3 polyunsaturated fatty acids in liver tissue of experimental of rats

(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; Oneway 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

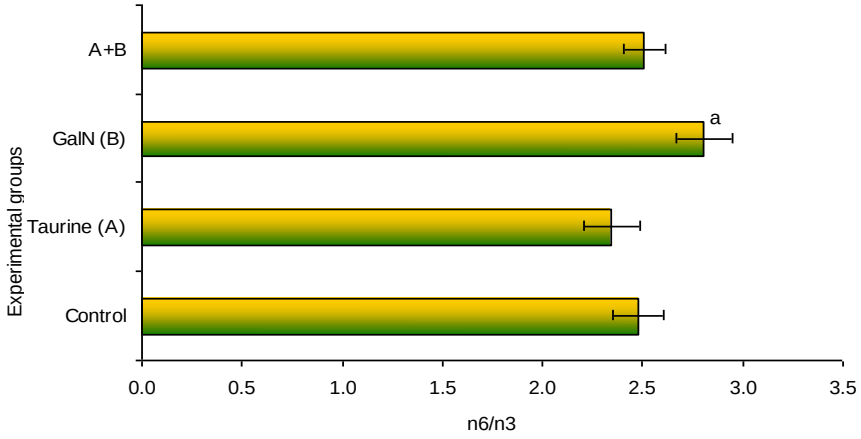


Fig 4.7.4.3 Ratio of n6 and n3 polyunsaturated fatty acids in liver tissue of rats

(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; Oneway ANOVA; SPSS version 10.

[a]$p<0.05$ significantly different compared with Group II taurine-administered animals

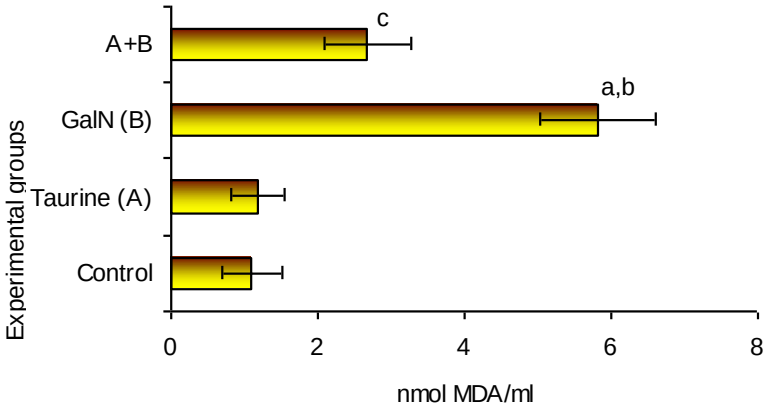


Fig 4.8.1.1 Level of lipid peroxides in plasma of control and experimental groups of rats

(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.

[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

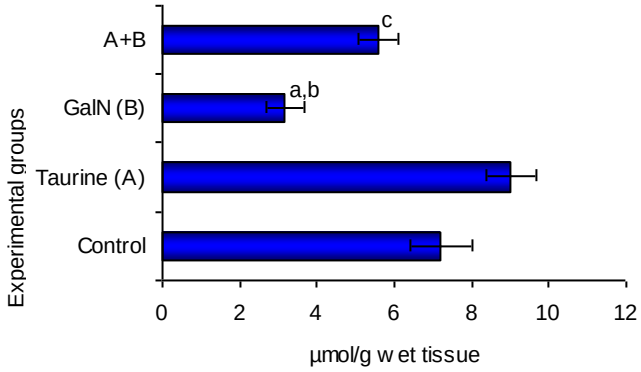


Fig 4.8.2.1 Level of reduced glutathione (GSH) in liver tissue of control and experimental rats

(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.

[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

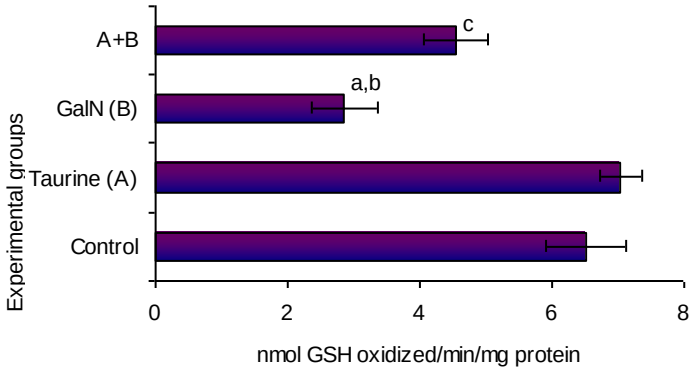


Fig 4.8.3.1 . Activity of glutathione peroxidase in liver tissue of control and experimental rats
(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 30 days

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

Results are mean ± SD for 6 animals; 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

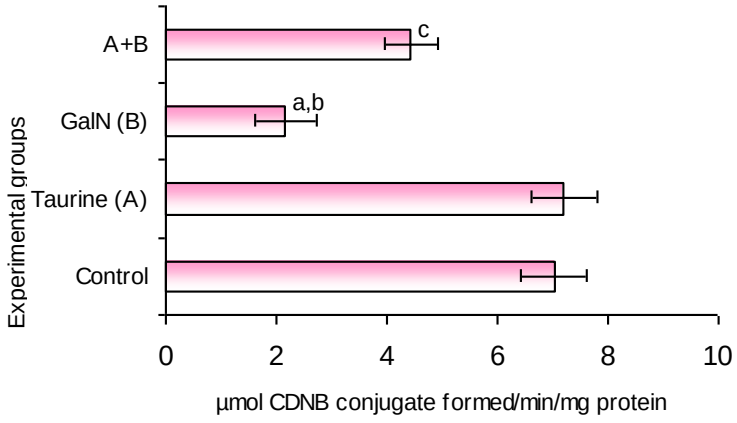


Fig 4.8.3.2 Activity of glutathione-S-transferase in liver tissue of control and experimental rats
(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 30 days

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

Results are mean ± SD for 6 animals; 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

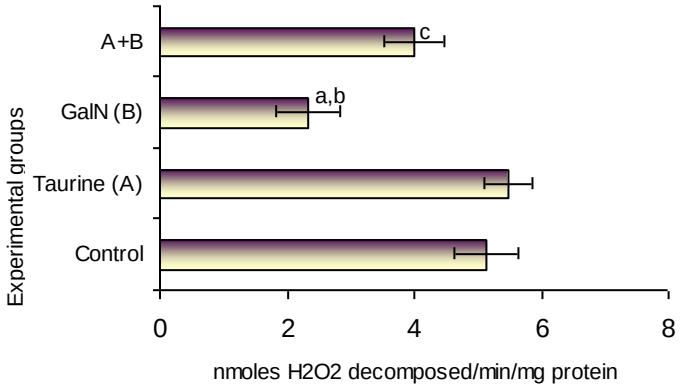


Fig 4.8.4.1 Activity of catalase (CAT) in liver tissue of control and experimental groups of rats
(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 30 days

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

Results are mean ± SD for 6 animals; 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

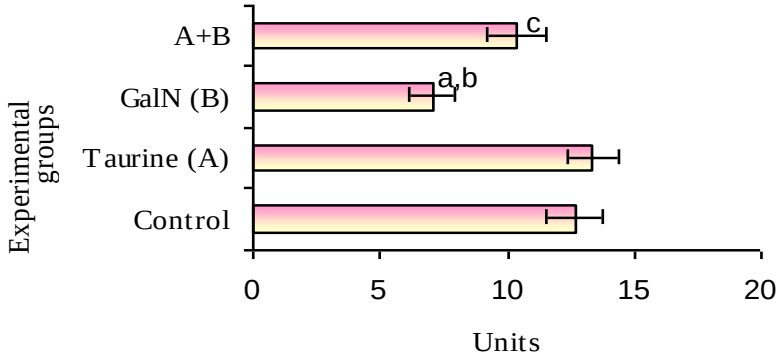


Fig 4.8.4. 2 Activity of superoxide dismutase in liver tissue of control and experimental rats
Unit: One unit is the amount of protein required to give 50% inhibition of adrenaline autoxidation.

(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; 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

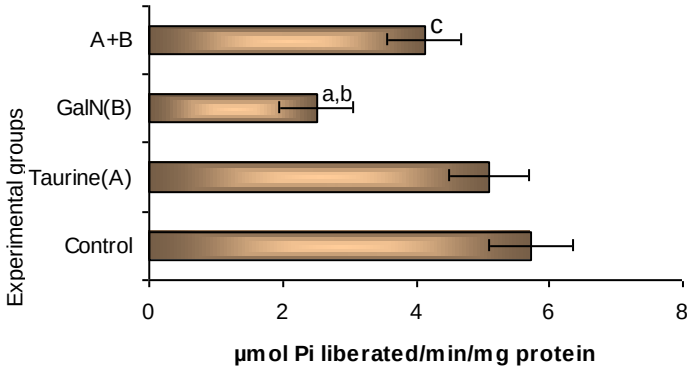


Fig 4.9.1 Activity of Total ATPase in liver tissue of control and experimental groups of rats

(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; 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

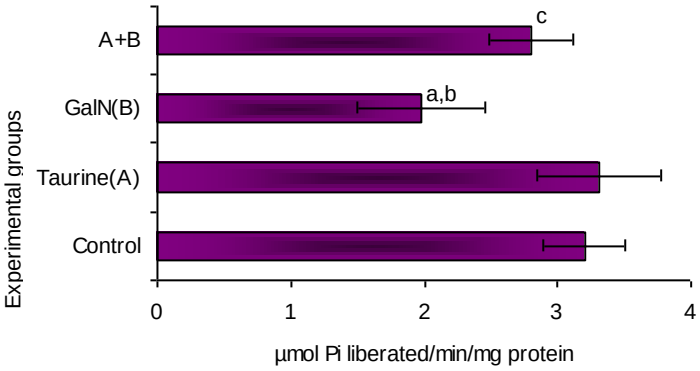


Fig 4.9.2 Activity of Na^+, K^+ -ATPase in liver tissue of control and experimental groups of rats

(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; 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

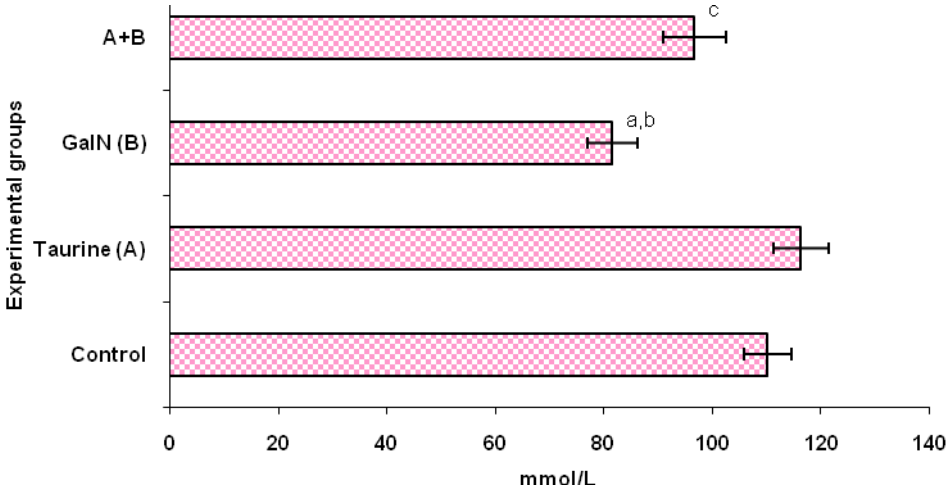


Fig 4.10.1 Levels of sodium in plasma of control and experimental groups of rats

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

(B): Galactosamine, 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 GalN -induced hepatic failure rats

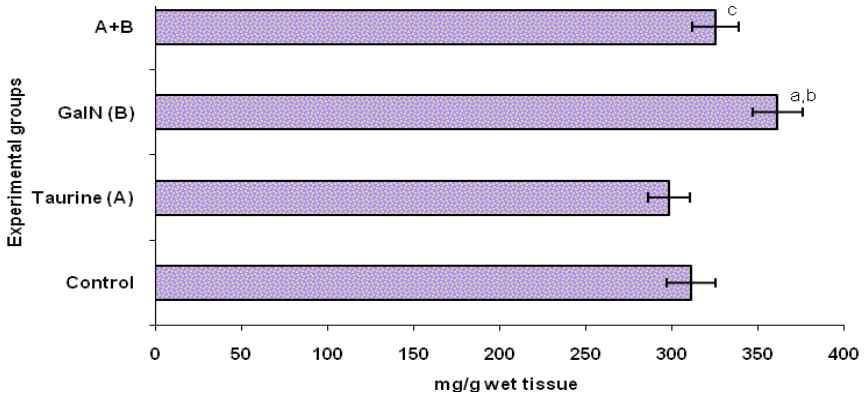


Fig 4.10.2 Levels of sodium in liver of control and experimental groups of rats

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

(B): Galactosamine, 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 GalN -induced hepatic failure rats

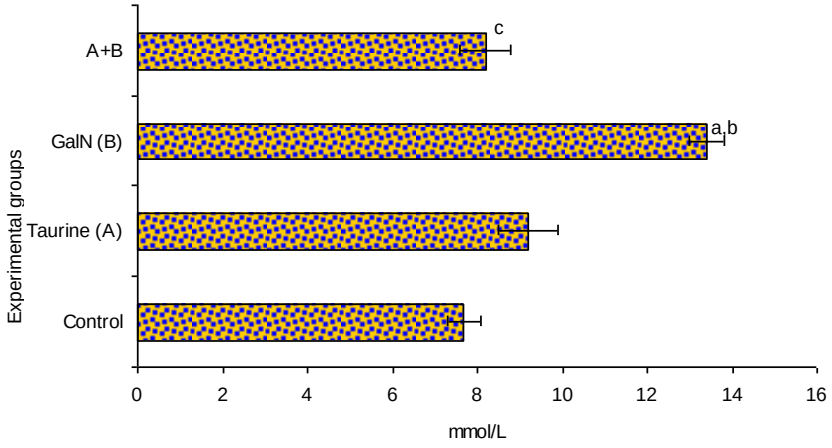


Fig 4.10.3 Levels of potassium in plasma of control and experimental groups of rats

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

(B): Galactosamine, 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 GalN-induced hepatic failure rats

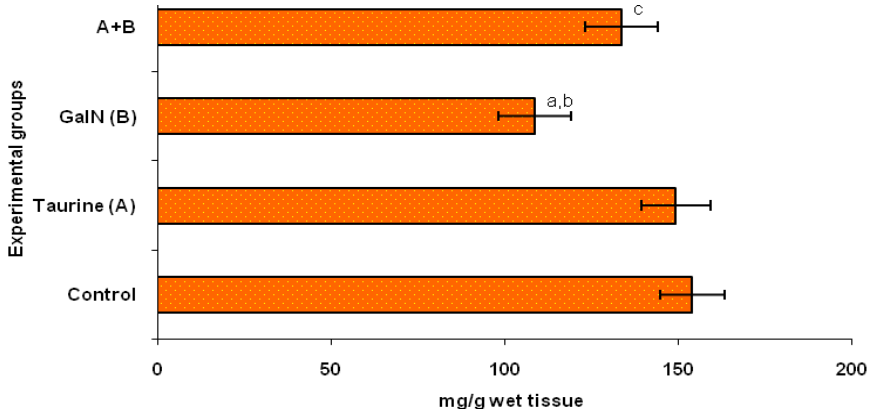


Fig 4.10.4 Levels of potassium in liver of control and experimental groups of rats

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

(B): Galactosamine, 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 GalN -induced hepatic failure rats

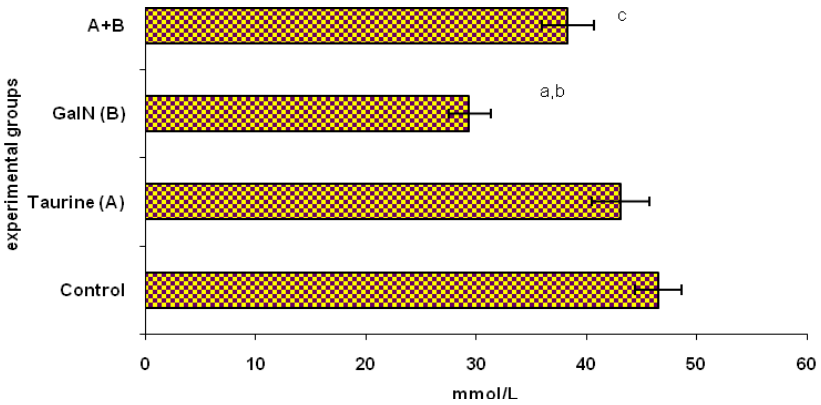


Fig 4.10.5 Levels of calcium in plasma of control and experimental groups of rats

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

(B): Galactosamine, 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 GalN -induced hepatic failure rats

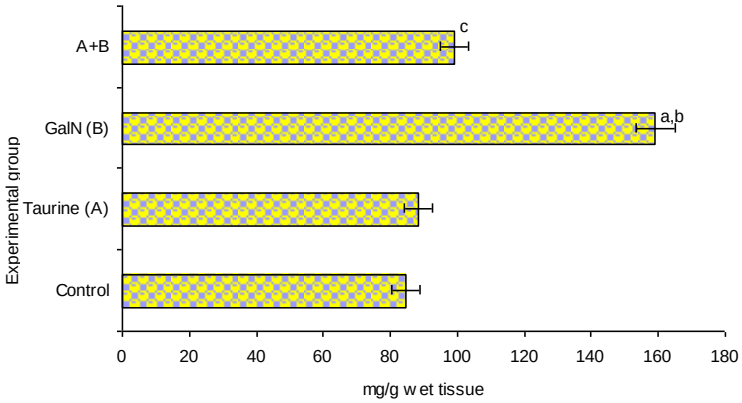


Fig 4.10.6 Levels of calcium in liver of control and experimental groups of rats

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

(B): Galactosamine, 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 GalN -induced hepatic failure rats

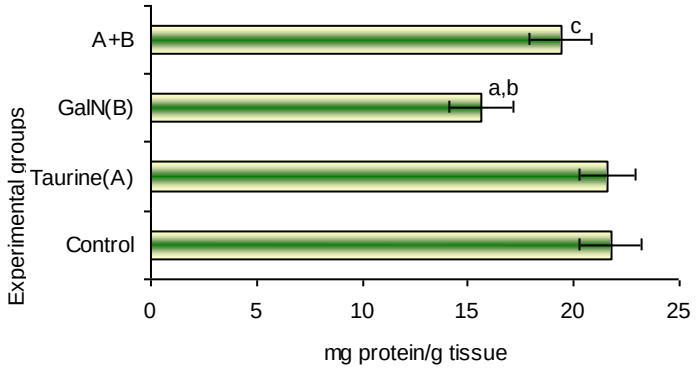


Fig 4.11.1.1 Level of protein in liver mitochondria of control and experimental groups of rats

(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; 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

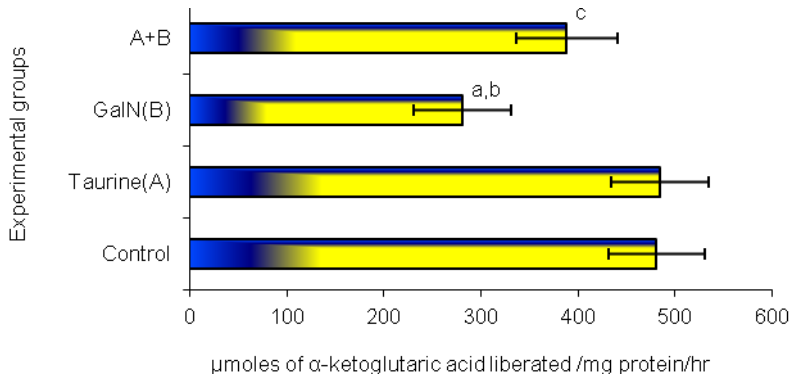


Fig 4.11.1.2 Activity of isocitrate dehydrogenase in liver mitochondria of rats

(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; 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

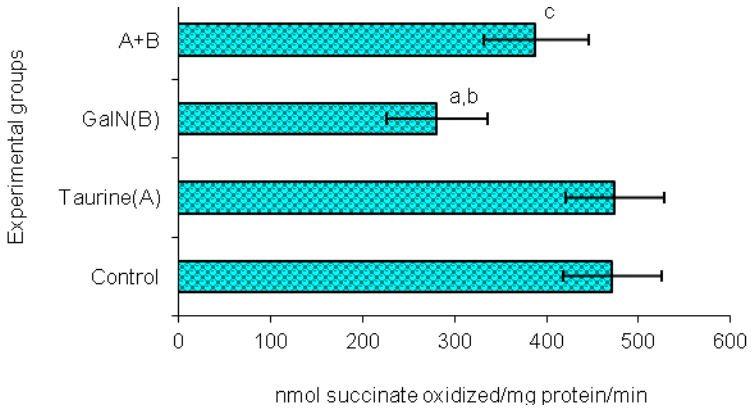


Fig 4.11.1.3 Activity of succinate dehydrogenase in liver mitochondria of rats

(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; 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

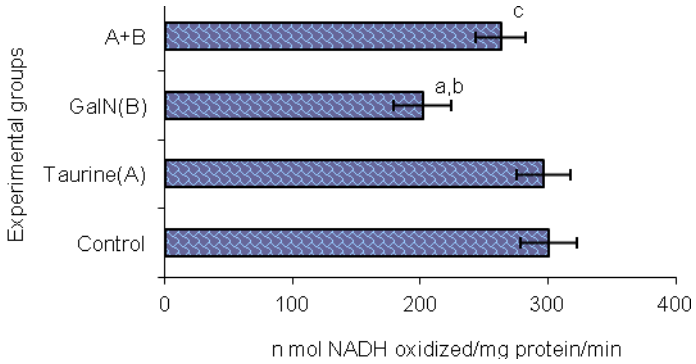


Fig 4.11.1.4 Activity of malate dehydrogenase in liver mitochondria of rats

(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; 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

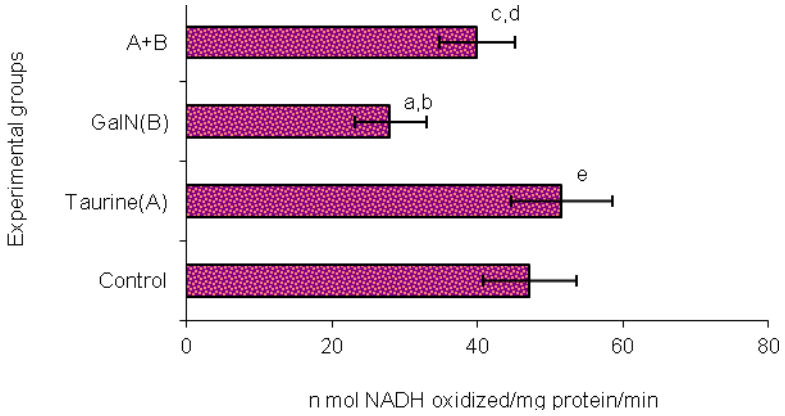


Fig 4.11.1.5 Activity of NADH dehydrogenase in liver mitochondria of control and experimental rats

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

(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

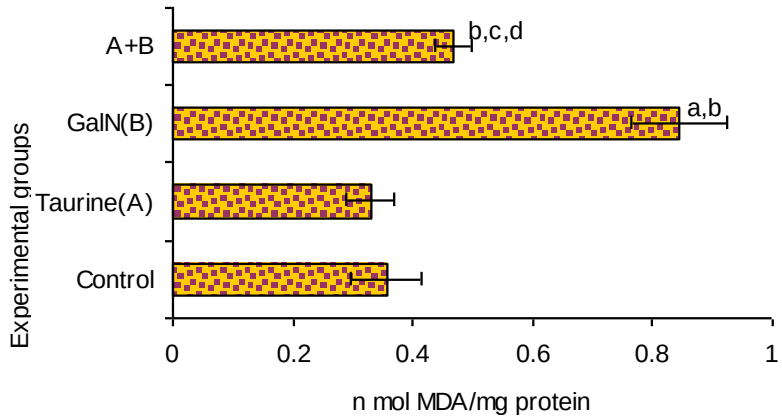


Fig 4.11.2.1 Level of lipid peroxides in liver mitochondria of control and experimental rats

(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; 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

[d] $p<0.05$ significantly different compared with Group I control animals

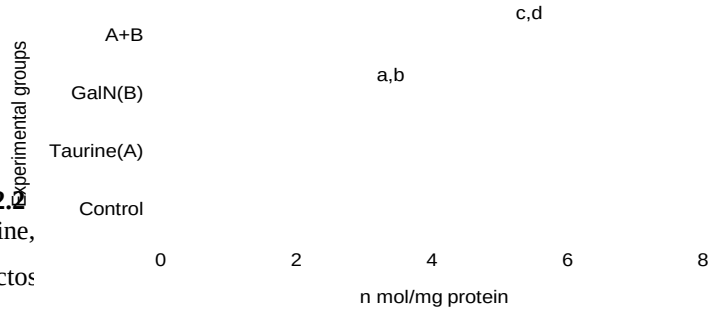


Fig 4.11.2.2 ats

(A): Taurine,

(B): Galactos

Resu 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<0.01 significantly different compared with Group II taurine-administered animals

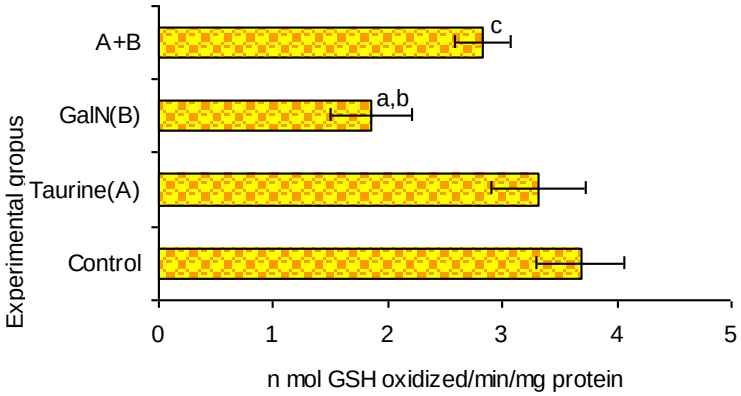


Fig 4.11.2.3 Activity of glutathione peroxidase (GPx) in liver mitochondria of rats

(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; 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

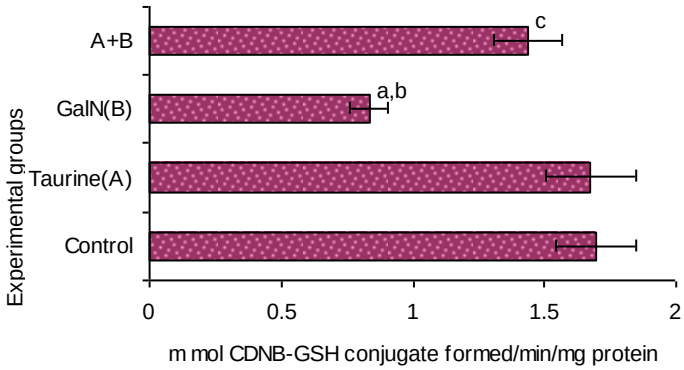


Fig 4.11.2.4 Activity of glutathione-S-transferase (GST) in liver mitochondria of rats

(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; 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

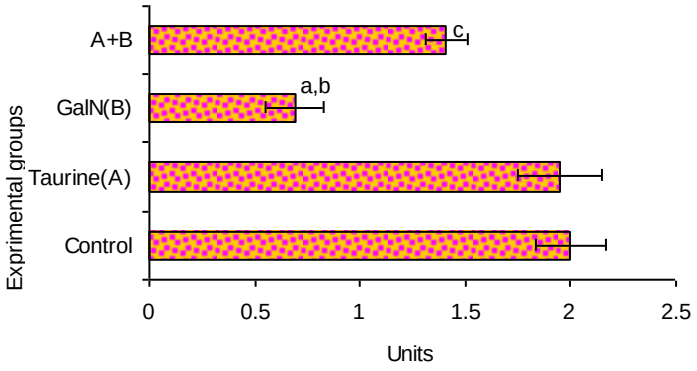


Fig 4.11.2.5 Activity of superoxide dismutase (SOD) in liver mitochondria of rats

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$100g^{-1}$ 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 rat

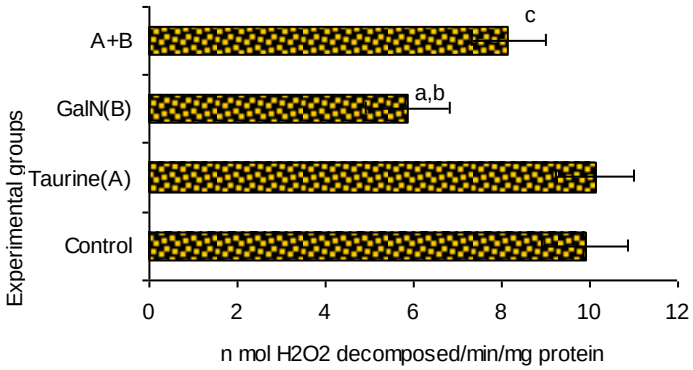


Fig 4.11.2.6 Activity of catalase (CAT) in liver mitochondria of control and experimental rats

(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; 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

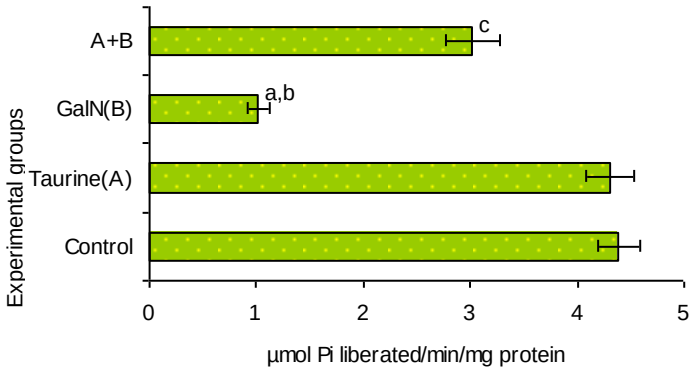


Fig 4.11.3.1 Total -ATPase in liver mitochondria of control and experimental groups of rats

(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; 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

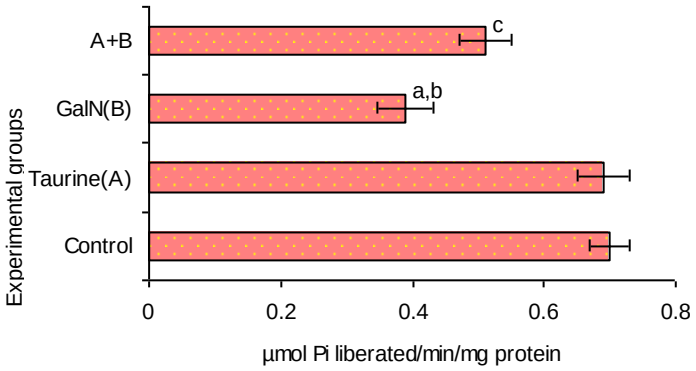


Fig 4.11.3.2 Ca2+ -ATPase in liver mitochondria of control and experimental groups of rats
(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 30 days

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

Results are mean ± SD for 6 animals; 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

SUMMARY & CONCLUSIONS

5. SUMMARY AND CONCLUSIONS

Fulminant hepatic failure (FHF) is a challenging syndrome in clinical medicine. Though it is not a very common disease, it is fatal and can affect even normally healthy people. Exact causes of the disease are not clearly known. But viral hepatitis and drug induced liver injury account for the majority of cases. Hepatitis E is a commonly encountered problem in the Indian sub continent affecting hundreds of thousands of people and resulting in high mortality. FHF is associated with clinical features like jaundice, shrunken liver, easy bruising, low levels of serum proteins, fatigue, multi-organ failure etc and metabolic derangements like hypoglycemia, hyperlipidemia, hyponatremia, defective protein synthesis, reduced energy production, decreased rate of urea production etc. These disturbances are predominantly attributed to oxidative stress, membrane destabilization and osmotic imbalances. The options available for patients suffering from this problem are minimal, liver transplantation being the main one. But due to cost, difficulties and inconveniences involved this procedure doesn't find favor among patients and care-givers. Use of cytoprotective and hepatoprotective drugs is considered a more acceptable alternative as a strategy to enhance liver regeneration. Isolated studies have suggested taurine, as a promising compound for this purpose. The present study was designed to investigate the hepatoprotective effect of this simple yet promising sulphur containing β-amino acid on experimentally induced FHF by feeding experiments using albino rats. D-GalN is known to induce FHF in rats. The protective effect of taurine, if any was studied in detail with induced FHF. The study shows that taurine supplementation can effectively retard or prevent all metabolic and structural aberrations associated with FHF. All relevant parameters were studied for this purpose. The conclusion was that consumption of foods rich in taurine like fish can be beneficial and supplementation of taurine in diet can help regenerate the damaged liver of patients suffering from this problem. Adult male Wistar strain albino rats, weighing 100-120g were selected for the

study. The animals were divided into four groups of six rats each. For each set of anlyses feeding trials with taurine were conducted at different times using four separate sets of albino rats. The quantity of taurine was decided after preliminary experiments using 25, 50, 75, 100, 150 and 200mg of taurine/kg body weight.

1. Group I was taken as the control fed on commercial feed.
2. Group II was fed on commercial feed with added taurine (100mg/kg body weight) to see if taurine by itself caused any undesirable changes.
3. Group III was fed on commercial feed alone. After 30 days feeding, rats were given i.p. injection of D-GalN (500mg/100g body weight/day for 2 days) for the induction of FHF to see the effects of induced-FHF.
4. Group IV was fed on commercial feed with added taurine and after 30 days they were given i.p.injection of D-GalN to see whether unlike in Group III the added taurine could prevent or lessen the adverse effects of D-GalN. All relevant biochemical, emzymic and histopathological parameters were also studied for this purpose.

Biochemical, enzymic and histopathological parameters studied:

- The effect of taurine on the liver parenchyma was studied by assaying the levels of **diagnostic marker enzymes like ALT, AST, LDH, arginase etc.**

- Prothrombin is a clotting factor synthesized by liver in whose absence or deficiency, clotting time or prothrombin time is prolonged. To study the effect of taurine on liver function, **prothrombin time** was measured.
- Liver is a site of bilirubin formation and urea cycle; to test taurine's effect on liver function, **serum bilirubin content and blood urea** were determined.
- The effect of taurine on **protein and glycoprotein content** of tissue and serum were studied.

- The effect of taurine on **glucose metabolism** with respect to important metabolic pathways *viz*. glycolysis, gluconeogenesis and glycogenolysis were also followed systematically.
- To evaluate the effect of taurine on **lipid metabolism** in induced FHF, levels of various lipid components and fatty acid profile were determined.
- In liver damage including human FHF and FHF induced by D-GalN, there is enhanced oxidative stress and significant decrease of antioxidant defense. Therefore the **anti-peroxidative effect and membrane stabilization action** of taurine were studied.
- Among the organelles, mitochondrial membrane damage causes the maximum harm to the cell. To study the effect of taurine on mitochondrial function the activities of **TCA cycle enzymes, respiratory marker enzymes, mitochondrial antioxidant defense system and membrane bound ATPases** were assayed.
- To confirm the protective action of taurine against D-GalN-induced FHF in rats the **histopathological pattern** was studied.

The hepatic damage induced by D-GalN is believed to be primarily due to the reactive oxygen species produced by activated hepatic macrophages. Defective protein synthesis also contributes to liver injury. In the present study D-GalN administration caused severe damage to the liver parenchyma, as evidenced by the rise in the liver specific enzymes in serum. Prothrombin time was prolonged. The contents of serum bilirubin and blood urea were markedly elevated. Due to defective protein and glycoprotein synthesis significant decrease occurred in the liver and serum content of protein and glycoprotein components. D-GalN caused metabolic disturbances chief among them were the changes that occurred in glucose and lipid metabolism. Hypoglycemia was a marked feature with other changes like reduced glycolysis, and gluconeogenesis contributing to the liver injury. Changes in lipid metabolism

included accumulation of fat, elevation of lipid parameters and unfavorable changes in the fatty acid profile. Also defective protein synthesis caused impairment in lipid bilayers formation that made cell and organelle membranes susceptible to oxidative stress. The antiperoxidative enzymes and reduced glutathione were markedly reduced and lipid peroxides were significantly elevated by D-GalN. Being the site of respiration, oxidative phosphorylation and generation of ATP, D-GalN-induced loss of mitochondrial membrane function resulted in inhibition of enzymes of TCA and ATP synthesis. Mitochondria are both source and easy targets of oxidative stress. Significant reductions were observed in antioxidant defense parameters and membrane transporters as a consequence of D-GalN toxicity in this study. Na^+ K^+ ATPase in the cell membrane and mitochondrial membrane play an important role in maintaining cell homeostasis. In the current study, loss of activity of Na^+ K^+ ATPase pump and also Ca^{2+} and Mg^{2+} dependent pumps due to membrane destabilization resulted in significant and cell death-inducing alterations in mineral (Ca^{2+}, $Na^{+,}$ K^+ and Mg^{2+}) balance. D-GalN intoxication also caused extensive hepatocyte necrosis and inflammation as evidenced by histopathology. The extensive hepatocyte parenchymal damage and membrane destabilization by D-GalN caused liver specific enzymes to spill into the blood stream as supported by their enhanced activity in serum.

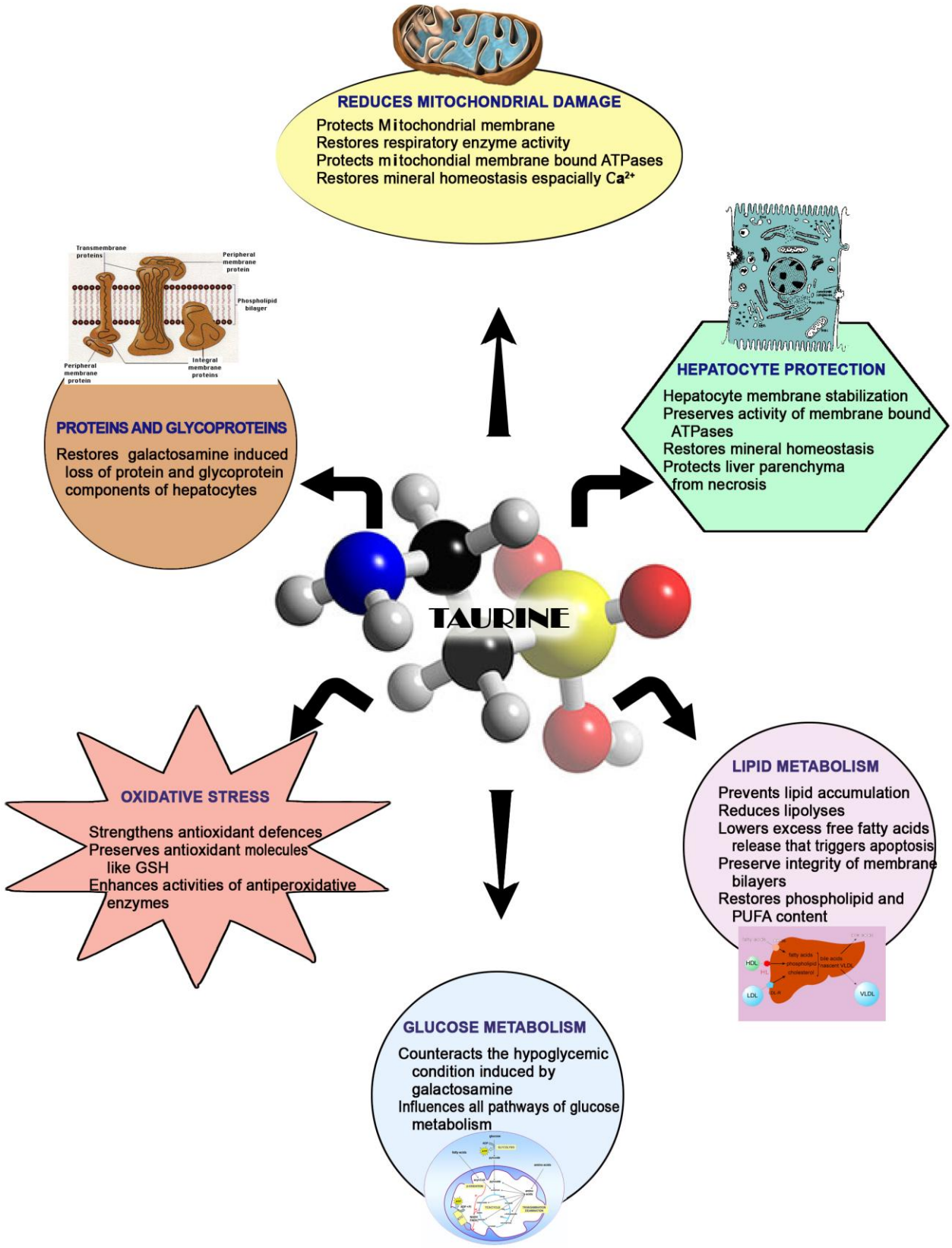
In the present study prior administration of taurine prevented the rise in the levels of liver specific enzymes in serum, which indicate that the histopathological alterations and the parenchymal damage induced by D-GalN in the hepatocytes were opposed by taurine. Histopathological analysis shows that taurine has prevented the parenchymal damage to liver tissue that was caused by D-GalN. Also the blood levels of urea, serum bilirubin and prothrombin time returned to normal which are indications of normal liver function. In the present study taurine administration in D-GalN-intoxicated rats alleviated the D-GalN-induced decreases in protein and glycoprotein content. Taurine helped to regain glucose

homeostasis by affecting and modulating changes in metabolic pathways of glycolysis, gluconeogenesis and glycogenolysis. Hypoglycemia was corrected by reducing glycogenolysis and enhancing gluconeogenesis and as a consequence glycolysis was restored. Taurine supplementation has helped to restore the lipid content to normal as indicated by the changes in levels of the parameters measured in liver and serum. One of the serious aberrations seen in D-GalN intoxication was loss of membrane intergrity that made cell and organelle membranes susceptible to attack by ROS. Taurine prevented the loss of membrane function and ameliorated oxidative stress. Lipid bilayers were stabilized by taurine that contributed to preserving the structure and function of several membrane bound proteins like the Na^+ K^+ ATPase pump and also Ca^{2+} and Mg^{2+} dependent pumps. Mineral homeostasis was restored that was unfavorably affected in D-GalN toxicity. Mitochondria are the powerhouses of the cell and they have a major role to play in enhancing cell death by either necrosis or apoptosis. Proper mitochondrial function is essential for the survival of the cell, they being the site of TCA cycle, respiration, Ca^{2+} sequestration and apoptosis. Taurine enhances the activity of TCA cycle enzymes and oxidative phopshorylation that restores the energy levels (ATP) in the cell. Calcium concentration is under strict control in the cells and mitochondria play a key role in eliminating calcium form inside of a cell. In D-GalN toxicity, oxidative damage to the membranes causes calcium to flood the cell triggering apoptosis and necrosis. Taurine plays an important role in stabilizing the membranes and prevents accumulation of excess calcium in cells.

Taurine is involved in a number of crucial physiological processes. Earlier studies demonstrate that pathology develops if the animal is depleted of taurine stores either through a taurine deficient diet or use of taurine transport antagonists. There is considerable scientific evidence concerning the pharmacological significance of taurine in maintaining the integrity

of organism, and this study further proves the therapeutic nature of taurine. These findings have considerable clinical significance and warrant further detailed investigation.

Earlier this century, Thomas A. Edison predicted "the doctor of the future will give no medicine, but will interest his patients in the care of the human frame, in diet, and in the cause and prevention of disease." In the years ahead, physicians and patients alike should embrace Edison's prediction and look to natural sources for healing and wellbeing.



SCHEMATIC DIAGRAM SHOWING PROBABLE MECHANISM OF ACTION OF TAURINE IN ATTENUATING GALACTOSAMINE-INDUCED HEPATIC FAILURE

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