Chapter 1

INTRODUCTION

Diabetes Mellitus (DM) is a genetically heterogeneous disorder but exhibits common features of glucose intolerance and hyperglycemia (Martin et al, 1992) along with abnormalities in lipid metabolism and oxidative stress (Islam and Loots, 2007). Hyperglycemia is a common characteristic of diabetes and is thought to result from a lack of insulin production by the pancreatic β-cells or the inability of the body to efficiently use the insulin that is produced. While diabetes may result from different origins, it is generally classified as insulin-dependent or type I diabetes mellitus (IDDM), non insulin-dependent or type II diabetes mellitus (NIDDM) and gestational diabetes (Amos et al, 1997 and Metzger et al, 1998).

The characteristic changes occurring in uncontrolled diabetes include a rise in blood glucose, increase in glycogen breakdown, gluconeogensis, fatty acid oxidation, ketone body production and urea formation. There is depression in the synthesis of glycogen, lipid and protein in the cells of those tissues that are normally dependent on insulin. Diabetes is thought to be fatal without regular insulin injections and even with insulin therapy, numerous life-threatening complications can result in these patients, such as cardiovascular disease, neuropathy, retinopathy, and kidney failure (Rabinovitch et al, 1992). Diabetes mellitus affects carbohydrate, fat and protein metabolism as well as affects the function of other organs (Dhawan and Pathak, 1999 and Noor et al, 2008). Hyperlipidemia, one of the common complications of diabetes mellitus is found in about 40% of diabetic (Abrams, 1982). The elevation in lipoproteins was shown to accelerate athrogenesis in diabetes mellitus (Howard, 1987). Diabetes is known to produce substantial changes in intracellular metabolism in most tissues, including liver (Satav et al, 2004; Sajad et al, 2008). In diabetes mellitus, attention has long been centered on the liver because of the importance of this organ in carbohydrate metabolism and regulation of blood sugar. Many studies (Nanji et al, 1986; Hamilton et al, 1987) revealed the

occurrence of hepatic changes in some cases of diabetic patients (Cefalu et al, 1991) and mice (Kume et al, 1994).

Development of diabetic complications has been hypothesized to be accelerated by generation of free radicals in cells and tissues (Baynes et al, 1991; Kennedy et al, 1997). At the molecular level, patients with diabetes often have high levels of intracellular oxidative stress as a result of hyperglycemia (Nishikwa et al, 2000; Rosen et al, 2001 and Singh et al, 2001). Since, glucose in a cell-free system can be auto-oxidized under physiological conditions via enediol tautomer formation which generates hydrogen peroxide; reactive intermediate such as hydroxyl and superoxide radicals, and ketoaldehydes (Brownlee et al, 1988 and Ceriello et al, 1996). Several studies have reported that glucose auto-oxidation can actually occur and could be responsible for increased oxygen radicals in diabetes (Baynes, 1991and Santini, 1997). High levels of reactive oxygen species are often present as a result of a reduced oxidant defense system (Maritim et al, 2003 and Bonnefont-Rousselot et al, 2000).

Increased oxidative stress has been reported to reduce insulin secretion and increase insulin resistance in certain diabetic models, thereby playing a possible role in the pathogenesis of diabetes (Suzuki et al, 1996). Production of the high energy compounds that fuel the biochemical, biophysical and mechanical functions of the body are associated with ongoing generation of potentially cytotoxic reactive oxygen species (ROS). ROS can attack, denature or modify structural and functional molecules and thereby cause cytotoxicity, tissue injury and dysfunction. In addition, ROS avidly react with nitric oxide (NO), which is a major signaling molecule with diverse biological functions. The ROS are highly reactive and potentially damaging to biomolecules, but are also required, at low concentrations, for acting as second messengers, gene regulators, and even helps in insulin signaling (Cai, 2006).

Diabetes, are characterized by inflammation (cytokines) and oxidative stress, due to disruption of the equilibrium between production of free radicals and their scavenging by multiple antioxidant systems (Zeyda et al, 2009 and Kanteo et al, 2010). Several reports underline the alterations of antioxidant micronutrient status in subjects with type I or II DM (Strain, 1991 and Walter et al, 1991). Alterations of metabolic processes in

diabetes also influence enzymatic defenses, and these changes may be associated with late complications of diabetes.

Antioxidant enzymes primarily account for intracellular defense, while several non-enzyme molecules, small molecule weight antioxidants, protect various components against oxidation in plasma. Intracellular antioxidant defense is primarily provided by antioxidant enzymes, which catalyze decomposition of reactive oxygen species. The three major antioxidant enzymes are Superoxide dismutase (SOD), Glutathione peroxidase (Gpx), and Catalase (CAT). It is maintained that high plasma lipid peroxides (LPO) in diabetes may result from oxidative destruction of erythrocyte membrane lipids (Uzel et al, 1987). Glutathione (GSH) acts as a direct scavenger as well as a co-substrate for Glutathione peroxidase. It is a major intracellular redox tampon system. Decrease in GSH levels and increase in LPO levels is the direct measure of increased oxidative stress. Elevated oxidative stress in diabetes plays an important role in the pathogenesis of diabetic complications (Baynes, 1991).

Natural defense against oxidative stress and antioxidants Reactive species can be eliminated by a number of enzymatic and nonenzymatic antioxidant mechanisms. SOD immediately converts O^{2-} to H_2O_2, which is then detoxified to water either by catalase in the lysosomes or by Glutathione peroxidase in the mitochondria. Another important enzyme is glutathione reductase. This enzyme regenerates glutathione which is used as a hydrogen donor by glutathione peroxidase during the elimination of H_2O_2. Diabetes has multiple effects on the activity of antioxidant enzymes, which further augment oxidative stress by causing a suppressed defense response (Aydin et al, 2001 and Wohaieb and Godin, 1987). Antioxidant treatments, such as vitamin E (Chung et al, 1999), vitamin C (Paolissso et al, 1994) and lipoic acid (Jain et al, 2000), are able to improve insulin action. Zinc also is involved in diabetes, and metabolic disorders of diabetes are associated with a depleted zinc status (Chausmer, 1998). Correction of Zn deficiency in subjects with type 1 DM leads to decreased lipid peroxidation (Faure et al, 1995; Goel et al, 2005) and improvements in glucose homeostasis (Faure et al, 1993).

Both type I and type II diabetes have the same long-term complications (Wilson et.al, 2003; Li et al, 2004; Song et al, 2005). Currently, mechanisms for the onset of

diabetes and the development of diabetic complications remain under extensive investigations. One of these mechanisms is abnormal homeostasis of trace elements. The presence of hyperzincuria, in addition to the observed lowered intestinal absorption of Zn, in both diabetic animals and humans have prompted speculation that diabetics are more susceptible to Zn deficiencies (Escobar et al, 1995). Zinc (Zn) plays several roles in insulin structural conformation, storage and secretion from the pancreas, and in insulin signaling pathways (Song et al, 2005; Li et al, 2007). Epidemiological study has demonstrated that exposure to a low concentration of Zn in drinking water was associated to an increased risk for the onset of type 1 diabetes (Haglund et al,1996; Zhao et al, 2001). This suggests that reduced Zn status is associated with diabetes (Al-Maroof et al, 2006; Sun et al, 2009; Tallman et al, 1999) and studies using animal models also showed that various Zn chelators induce diabetes in some mammalian species, e.g., rabbits, mice, and hamsters, by β-cell destruction (Goldberg et al, 1990; Goldberg et al, 1991). It is now well evident fact that Zn deficiency significantly enhances the blood glucose level in diabetes-prone experimental animals (Kechrid et al, 2001).

There are at least more than 300 catalytically active Zn metalloproteins and more than 2,000 Zn- dependent transcription factors. Therefore, Zn is an integral component of a large variety of proteins and enzymes, and participates in a wide variety of metabolic processes including carbohydrate, lipid, protein and nucleic acid synthesis or degradation. Zinc is also required to maintain structural integrity, regulations of enzyme function, healthy immune system, proper wound healing, normal growth and development of an individual during pregnancy, childhood, and adolescence (Coleman, 1992).

Zinc has been implicated in the cellular oxidant status of people with diabetes. Because type I diabetes is thought to be the result of an autoimmune attack on the β-cell and the destruction of these cells results in less bioavailable zinc for use in important antioxidant enzymes. It has been proposed that the lack of zinc available for use in the enzymes may contribute to the tissue damage observed in diabetes (Rabinovitch et al, 1992 and Meyer et al, 2009). Because Zn has no storage form, there is need for a constant supply and Zn availability to cells is particularly well regulated, albeit poorly understood (Sekler et al, 2007). Epidemiological studies have demonstrated that exposure

to low concentrations of Zn in drinking water is associated with an increase in type I diabetes (Haglund et al, 1996 and Zhao et al, 2001).

Metallotheionin (MT) plays a role in the homeostasis of Zn and has found to alter in diabetic state (Li et al, 2007). Under physiologic conditions, zinc-MT is the predominant form of the metal-binding protein (Kagi, 1991). MT, is an important component of the antioxidant protein pool of the cell and is an efficient radical scavenger due to the presence of cystein residues, coordinated by Zn(II) and/or Cu(I) ions (Binz and Kagi, 1997). The major roles of MTs are for detoxification of heavy metals, homeostatic regulation of essential metals, and protection of tissues against various forms of oxidative injury (Koropatnick and Zalups, 2000). Diabetes also significantly impairs Zn homeostasis (Terres- Martos et al, 1998). Zinc as a trace element is an essential nutrient for human beings and has been found to be protective in some kind of liver injuries (Pathak et al, 2002, Sidhu et al, 2005; Goel et al, 2005).

Since Zn is a critical factor for many important proteins, enzymes and transcription factors so its deficiency may cause alterations in the function of these enzymes or proteins thereby leading to the initiation or acceleration of liver pathogenesis in the diabetic subjects. Zn is essentially considered non-toxic in human beings in prescribed dosages, and is additionally considered neither carcinogenic, nor mutagenic or teratogenic (Vallee et al, 1993) and thus it serves as an excellent candidate for further investigation for an intervention in diabetes.

Objectives:

The present study was planned to explore the efficacy of zinc in containing the biophysical and biochemical events leading to pathogenesis of liver as well as regulating the glucose levels during alloxan induced experimental diabetes with the following objectives:

- The experimental study aimed at investigating the serum changes in glucose, insulin and lipid profile in zinc treated normal and diabetic rats.

- Zinc homeostasis was studied by using biokinetics studies with ^{65}Zn and zinc status was evaluated in serum, liver and urine of different treatment groups.
- Oxidative stress was measured in liver by investigating the levels of Lipid peroxidation (LPO), Glutathione reduced (GSH), total Glutathione and activities of Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx) and Glutathione Reductase (GR) in liver.
- Carbohydrate metabolism was assessed in liver by evaluating the status of enzymes like Succinic Dehydrogenase (SDH), Lactate Dehydrogenase (LDH), Hexokinase, Glucose-6-phosphatse, Glucose-6-Phosphate isomerase and Glycogen Phosphorylase and levels of glycogen.
- Transport of glucose was assessed by ^{14}C-U-Glucose uptake and turnover of glucose was studied using radiorespirometry technique in liver slices. Also mRNA expression of Glut2 was investigated using RT-PCR.
- To understand the calcium homeostasis, calcium, cAMP, Ca^{2+} ATPase levels were carried out along with the study of ^{45}Ca uptake in liver of animals of different treatment groups.
- To evaluate the Na^{+}/K^{+} ATPase activity in liver of all the treatment groups.
- Liver damage was assessed by studying the activities of liver marker enzymes viz; Alkaline Phosphatase (ALP), Alanine Amiotransferase (ALT) and Aspartate Aminotranferase (AST).
- Hisotpathological studies were carried out on liver of animals using both light and electron microscopy.