REVIEW OF LITERATURE

2.0 Review of Literature :

2.1 General Aspects :

Diabetes is an age old disease. With its varied complications it spreads its domain to different discipline of medicine. There are many well known journals exclusively on diabetes. Literatures on diabetes are published in many other journals too. There are more than hundred thousand publications on diabetes. Some are on the clinical approaches to diagnose cases, some on therapeutic variations for successful control of blood sugar, many on the invention of drugs, some on the complications, some to find out the aetiological basis of diabetes etc. Our goal is to find out the relation of Selenium on the biochemistry of streptozotocin induced diabetic mice. so we have concentrated only on the different biochemical parameters which are affected in the diabetes process and as we have tested in experimentally induced mice the literatures surveyed by us are all for animals with drug induced diabetes.

The distribution of LDH-isoenzymes from homogenate-supernatant of muscle soleus of normal and streptozotocin-diabetic male rats was investigated by Wohlrab and Schmidt in 1975 by agargel-electrophoresis. Five LDH-isoenzymes could be detected in the muscle of normal rats. Diabetes was induced by intravenous injection of 65 mgm streptozotocin per kg body weight. After 1-2 month duration of diabetes the LDH 1 was decreased and the LDH 4 was increased. The H-subunit value was decreased. In long term diabetes (11 months) the values of single fractions and the H-subunit value did not differ from those of controls [59].

Armstrong et al in 1976 studied succinate dehydrogenase activities of skeletal muscles in normal and streptozotocin induced diabetic rats. Enzyme activities in all muscles declined to a lower final level and exhibited a more rapid decay in animals receiving the larger dosage, both diabetic and Karela juice fed rats. In addition they also observed that Karela does not always reverse the effects on drug-metabolising enzymes in STZ-induced diabetes [60].

Adipose lactate dehydrogenase (LDH) (EC 1.1.1.27) isozyme distribution was altered in streptozotocin diabetic and fasting rats resulting from a relative reduction of subunit A. Treatment with insulin for 2 days partially restored the relative content of isozyme 5 to control values in the diabetic rats, and the effect of insulin was not inhibited by simultaneous injection of actinomycin D or puromycin. When the epididymal adipose tissues isolated from control animals were incubated in vitro with dibutyryl adenosine 3', 5' -cyclic monophosphate or epinephrine, a relative decrease in subunit A was observed; whereas either compound caused an increase in subunit A in diabetic tissues. Chang and Rothrock in 1977 suggested that the redistribution of

LDH isozyme under these conditions is to prevent excessive accumulation of lactate in the tissue [61].

Studies were undertaken by Nakayama and Nakagawa in 1977 to examine cholesterogenesis in the intestine of streptozotocin-diabetic rats by measuring incorporation of [2(-14)C] acetate into cholesterol and 3-hydroxy-3-methylgultaryl CoA reductase (HMG-CoA reductase, EC 1.1.1.34) activity. In these diabetic rats, the intestinal mucosal weight and food consumption were markedly high. The incorporation of [2(-14)C] acetate into cholesterol was significantly increased in all diabetic intestinal segments. However, the rates of production of fatty acids and carbon dioxide were not affected. Hepatic HMG-CoA reductase activities were markedly reduced during both the diurnal high and low periods in these diabetic rats, and there was no diurnal variation. In contrast, the specific activities of this enzyme in jejunal crypt cells during both the diurnal high and low periods were significantly higher in these diabetic rats without loss of diurnal variation. Total reductase activity per segment of intestine in jejunal and ileal mucose (villi + crypt cells) was increased in these diabetic rats. Control rats had higher total and specific activity of ileal mucosal (villi + crypt cells) reductase than of jejunal mucosal reducatse during the diurnal high period. The jejunal-ileal gradient in reductase activity and the incorporation of [2(-14)C] acetate into cholesterol did not change significantly with streptozotocin-diabetic rats. The results indicate that in streptozotocin-diabetic rats, hepatic cholesterogenesis decreases but intestinal synthesis increases [62].

The activities of UDP glucuronosyltransferase, microsomal epoxide hydrolase and cytosolic glutathione S-transferase were measured by Rouer et al in 1981 in the liver of spontaneously (db/db and ob/ob) or streptozotocin-induced diabetic mice. An important (2-3-fold) increase of most phase II activities was observed in streptozotocin-treated animals, whereas slighter changes were detected in spontaneously diabetic animals. The latter also exhibited physico-chemical modifications of the liver microsomal membranes, as shown by the temperature-induced variations of epoxide hydrolase activity [63].

Lenzen and Panten in 1983 showed that succinate dehydrogenase activities in homogenates of rat and ob/ob mouse pancreatic islets were only 13% of the activities in homogenates of liver and were also several times lower than in homogenates of pancreatic acinar tissue. This indicates that the content of mitochondria in pancreatic islet cells is very low. The very low activity of succinate dehydrogenase is in agreement with the low mitochondrial volume in the cytoplasmic ground substance of pancreatic islet cells as observed in morphometric studies. This may represent the poor equipment of pancreatic islet cells with electron transport

chains and thus provide a regulatory role for the generation of reducing equivalents and chemical energy for the regulation of insulin secretion. The activities of succinate dehydrogenase in tissue homogenates of pancreatic islets, pancreatic acinar tissue and the liver were significantly inhibited by malonate and diazoxide but not by glucose, mannoheptulose, streptozotocin or verapramil [64].

Verschoor et al have previously suggested that mechanisms other than reduced lipoprotein lipase (LPL) activity might contribute to the defect in plasma removal of very low density lipoprotein (VLDL)-triglyceride (TG) observed in insulin-deficient rats. To further evaluate this phenomenon, removal rates of TG in nonfractionated plasma, as well as in isolated lipoprotein fractions obtained from insulin-deficient and control rats, were compared in a new, sensitive in vivo bioassay system (estradiol-treated male rats with a consistently low endogenous VLDL-TG pool). Removal of TG in nonfractionated plasma from insulin-deficient rats was slower than that of control rats: 3.0 +/- 0.3 vs 1.6 +/- 0.2 min (P less than 0.001). No difference was found in removal rate of isolated VLDL-TG (2.5 +/- 0.3 vs 2.6 +/- 0.4 min), or in removal rates of TG carried in other lipoprotein fractions. Authors in 1984 determined the effect of injection into normal rats of aliquots of dialyzed lipoprotein-free (D greater than 1.215) plasma from insulin-deficient and control rats on the removal rate of normal VLDL-TG, and found that lipoprotein-free plasma from insulin-deficient rats significantly (P less than 0.01) prolonged removal of normal VLDL-TG (4.3 +/- 0.4 to 6.8 +/- 0.7 min). This same fraction did not interfere with the in vitro hydrolysis of normal VLDL-TG by post-heparin LPL. Thus, a factor in the D greater than 1.215 plasma fraction of insulin-deficient rats is present which interferes with the rate of removal of TG from plasma, unrelated to inhibition of LPL activity [65].

Agius and Gidari in 1985 demonstrated that Streptozotocin (STZ) increased the activity of mouse hepatic glutathione (GSH) S-transferases assayed with 1-chloro-2,4-dinitrobenzene. Nicotinamide administered prior to STZ prevented the hyperglycemia indicative of STZ-induced diabetes, but had no effect on the increase in GSH S-transferase activity caused by the drug. Another diabetogenic agent, alloxan, did not alter GSH S-transferase activity. Thus, streptozotocin may be increasing GSH S-transferase activity directly, and not as a result of the diabetic state the drug induces. Two transferases were characterized from mouse liver cytosol. One was a homodimer with a subunit molecular weight of about 28,000 and a pI of about 8.2. The other was also a homodimer with a subunit molecular weight of about 27,500 and a pI of about 9.2. The pI 8.2 GSH S-transferase was induced by STZ, while the pI 9.2 transferase was decreased by the drug. At least one other transferase appeared to be induced by STZ.

Two other nitroso compounds, chlorozotocin and diethylnitrosamine, also increased GSH S-transferase activity, suggesting that this effect may be nitroso related [66].

The activities and zonal distribution of key enzymes of carbohydrate metabolism were studied in livers of diabetic rats by Miethke et al in 1985. 48 h after alloxan treatment the following alterations were observed, intermediate values being reached after 24 h: Blood glucose, acetoacetate and beta-hydroxybutyrate were increased to more than 500%; liver glycogen was reduced to about 10%. Portal vein insulin was reduced to below 10%, portal glucagon was increased to almost 200%. The glucogenic enzymes phosphoenolpyruvate carboxykinase and glucose-6-phosphatase were enhanced to 320% and 150%, respectively. The glycolytic enzymes glucokinase and pyruvate kinase L (differentiated from the M2 isoenzyme with a specific anti-L-antibody) were lowered to 50% and 75%, respectively. The citrate cycle enzyme succinate dehydrogenase remained unchanged. The normal periportal to perivenous gradient of phosphoenolpyruvate carboxykinase of about 3:1, as measured in microdissected tissue samples, was enhanced to about 4:1 with activities elevated to 230% and 190%, respectively, in the two zones. The normal periportal to perivenous gradient of pyruvate kinase L of about 1:1.7, as determined with the microdissection technique, was reduced to about 1:1.4 with levels lowered to 55% and 45%, respectively, in the two zones. The even zonal distribution of pyruvate kinase M2 remained unaltered [67].

Favreau and Schenkman in 1987 measured the cytochrome P-450 dependent hydroxylation of testosterone in hepatic microsomes of control, diabetic and insulin treated diabetic rats. The observed decrease in testosterone 16α-hydroxylase activity in diabetes, an activity previously shown to be largely due to RLM 5, was accompained by a dramatic decrease in immuno detectable RLM 5. Diabetic rats which received insulin had elevated testosterone 16α-hydroxylase activity relative to the diabetic animals, which was accompained by a corresponding increase in the levels of RLM 5. These results provide evidence that specific constitutive cytochrome P-450 enzymes are altered in the diabetic state and that these changes are not permanent since they can be overcome, at least partially, by insulin replacement therapy [68].

These studies have been carried out by Golay et al in 1988 in rabbits with alloxan-induced diabetes in order to see if insulin deficiency affects low density lipoprotein (LDL) catabolism. The results showed that plasma LDL-cholesterol was lower in diabetic rabbits, associated with a fall in the cholesterol to protein ratio of LDL particles. In addition, 125I-LDL disappeared more slowly from plasma of diabetic rabbits, leading to a significant reduction in

fractional catabolic rate and a decrease in residence time of 125I-LDL. These data demonstrated that LDL composition and catabolism are greatly altered as a consequence of insulin deficiency [69].

Monospecific polyclonal antibodies to five constitutive hepatic microsomal cytochromes P-450 were prepared by Favreau and Schenkman in 1988. These antibodies were used to monitor alterations in the content of the enzymes in livers of diabetic male rats. Within 3 wk of onset of streptozocin induced diabetes, immunodetectable levels of RLM3 and RLM5 were decreased by 85 and greater than 95%, respectively. Insulin treatment for 1 wk reversed the decline in these isozymes and restored RLM3 to 60% and RLM5 to 53% of levels found in the untreated rat. After a 2nd wk of therapy, these levels were returned to 86 and 92%, respectively. In contrast, the levels of RLM5b and RLM6 were elevated in diabetes 1.7- and 8-fold, respectively. Insulin treatment for 1 wk only slightly decreased the levels of RLM5b but completely reduced RLM6 levels to those seen in age-matched untreated rats. After the 2nd wk of insulin treatment, the level of RLM5b was almost completely restored to normal, with no additional change in the RLM6 level. The level of a fifth enzyme, RLM5a, was not markedly altered by diabetes or by insulin treatment. The results suggest there are at least three types of responses by constituents of the cytochrome P-450 population to diabetes: no change in the microsomal content, a rapid increase when insulin level declines and restoration when insulin is supplied and a rapid decline when insulin level declines and a restoration by insulin treatment [70].

In the present investigation by Godin et al in 1988, it is shown that rats made diabetic with alloxan, an agent differing from streptozotocin both chemically and its mechanism of diabetogenesis, show virtually identical tissue antioxidant enzyme changes, which as is the case with streptozotocin, are preventable by insulin treatment. The finding that the patterns of antioxidant enzyme alterations in chemically induced diabetes are independent of the diabetogenic agent used and the presence of similar abnormalities in tissues of spontaneously diabetic (BB), Wistar rats (particularly when diabetic control is less than optimal) suggest that the changes observed are a characteristic feature of the uncontrolled diabetic state and that these may be responsible for (or predispose to) the development of secondary complications in clinical diabetes. The study also showed increased in glutathione reductase activity, decreased susceptibility to oxidative glutathione depletion and an increased production of malondialdehyde (an indirect index of lipid peroxidation) in diabetes. The extent of this increase in susceptibility of red cell lipids to oxidation paralleled the severity of diabetic complications. Authors view is

that increased oxidative activity may play an important role in the pathogenesis of complications associated with the chronic diabetic state [71].

Thomas et al in 1989 determined the activities of peroxisomal β-oxidation, cytosolic and microsomal epoxide hydrolase as well as soluble glutathione-s-transferases in the livers of alloxan and streptozotocin-diabetic mice. After initiation of diabetes serum glucose levels were elevated more than the increase in the activities of peroxisomal β-oxidation and cytosolic epoxide hydrolase. The activities of microsomal epoxide hydrolase and glutathione-s-transferase were reduced to about 71% and 80% of controls. Application of depot insulin twice a day for 10 days restored the initial glucose levels and enzyme activities except for peroxisomal β-oxidation. Starvation similarly resulted in increase in peroxisomal β-oxidation and cytosolic epoxide hydrolase activity. Microsomal epoxide hydrolase was significantly decreased whereas glutathione-s-transferase was only marginally reduced. Except for glutathione-s-transferases initial enzyme activities were restored upon refeeding within 10days. With this the authors predicted that this may indicate that high levels of free fatty acids or their metabolites which are known to accumulate in liver in both metabolic states may act as endogenous peroxisome proliferators [72].

Bollen et al in 1990 showed that isolated hepatocytes from streptozotocin-diabetic rats failed to respond to a glucose load with an activation of glycogen synthase. This lesion was associated with severely decreased activities of glycogen-synthase phosphatase and of glucokinase. All these defects were abolished after consumption for 13-18 days of drinking water containing Na_3VO_4 (0.7mgm/ml), and they were partially restored after 3.5 days, when the blood glucose concentration was already normalized. In all conditions the maximal extent of activation of glycogen synthase in cells closely parallelled the activity of glycogen-synthase phosphatase [73].

The effect of insulin-dependent diabetes on the hepatic microsomal activity of cytochrome P450 III and P 450 IV family proteins was investigated in rats pretreated with streptozotocin by Barnet et al in 1990. It was concluded that insulin-dependent diabetes induces proteins of the P 450 III and P 450 IV families and that the hyperketonaemia that accompanies diabetes is largely responsible for the changes in the latter family [74].

The effect of oral administration of sodium ortho vanadate for 5 weeks on hepatic glycogen metabolism was studied in control and streptozotocin induced diabetic rats by Pugazhenthi and Khandelwal in 1990. Diabetes caused hyperglycaemia (5-fold increase), hypo insulinemia (85% decrease), and hyperglucagonemia (4 fold increase). There were also marked decrease in liver

glycogen and activities of glycogen-metabolizing enzymes in liver. Although vanadate administration in control animals showed no significant effect on the various parameters measured except for a 70% decrease in plasma insulin, this treatment in diabetic rats restored these parameters to near control values. In conclusion the authors depicted an insulin like in vivo action of vanadate on various parameters related to hepatic glycogen metabolism [75].

Donahue and Morgan in 1989 showed the ability of sodium metavanadate to reverse the effects of streptozotocin induced diabetes on hepatic cytochrome P-450 isozymes in male rats. Streptozotocin caused P-450 h levels to fall 95%, and P-450 j and P-450 b levels to rise 8 and 40-fold, respectively, after 1 week. Furthermore, P-450 h m RNA levels correlated well with levels of P-450 h apoprotein for all treatment groups, indicating that P-450 h suppression in diabetic rats is under pretranslational control and is independent of the increased expressions of P-450 j and P-450 b, and of the hyperlipidemia and ketosis that occurs in diabetes. Vanadate is capable of separating the effects of diabetes on expression of individual P-450 isozymes [76].

Dumingo et al in 1990 in a letter to the editor pointed out that increased levels of vanadium in several tissues after orthovanadate (50 ppm v) or sodium metavanadate (50 ppm $NaVO_3$) administration. Signs of renal toxicity were also observed at 50 ppm $NaVO_3$. Moreover, ammonium metavanadate decreased erythropoiesis and maturation of erythrocytes when given to rats in drinking water for a period of 2, 4 or 8 weeks [77].

This study was performed by Jain et al in 1990 to determine whether or not hyperglycaemia in diabetes results in elevated levels of lipid peroxidation products in red blood cells (RBC). Diabetes was induced in rats by treatment with streptozotocin. The level of lipid peroxidation products was examined in fresh RBC by measuring their thiobarbituric acid (TBA) reactivity after 2 and 4 months of induction of diabetes. Hyperglycaemia was assessed by measuring the level of glycosylated hemoglobin and blood glucose. Results show that lipid peroxidation levels were significantly higher (50% to 84%) in RBC of diabetic rats than in controls. The increase in the level of lipid peroxidation was blocked in diabetic rats in which hyperglycaemia was controlled by insulin treatment [78].

A week after application of alloxan (200 mgm/kg. s.c.) Lackovic and Salkovic in 1990 found the concentration of serotonin, dopamine and norepinephrine to be increased in the brain of a diabetic rat. Accumulation of these monoamines, produced by inhibition of monoamine oxydase with pargyline (100 mgm/kg i.p.) suggesting a decrease in deamination rate. Surprisingly, however, after an intracerebro-ventricular administration of non-diabetogenic doses

of streptozotocin (5-20 mgm/kg) or alloxan (20 mgm/kg), changes in brain monoamines were similar to those observed in diabetic animals. This observation apparently suggests that the CNS effect of streptozotocin or alloxan is not necessarily related to a diabetogenic, beta-cytotoxic action of these substances [79].

Dash et al in 1991 found hyperglycemia due to experimental diabetes induced in rats, causes a decrease in the activity of Acetylcholinesterase in brain regions and heart; changes in the heart being more significant than the brain. Insulin administration reversed this effect in both the heart and the brain. Significant increase in the levels of catecholamines were also found in the brain regions in diabetes, which was reversed by insulin. The decreased activity of acetylcholinesterase observed in diabetes may be due to an early impaired glucose oxidation and glucose transport as a result of lack of insulin, which causes specific alterations in neurotransmitter levels, thereby effecting blood brain barrier transport, thus causing brain dysfunction [80].

This is a report by Kondoh et al in 1992 investigating the methylglyoxal (MG) bypass in animals, by which D-lactate is produced from triosephosphate via MG. Rats were made diabetic using streptozotocin or starved for 72 h. D-Lactate and various metabolites related to it, such as L-lactate, pyruvate, methylglyoxal, glucose and inorganic phosphate, were measured in the blood plasma, liver and skeletal muscle of the rats. Diabetic and starved rats had significantly higher levels of D-lactate in plasma, liver and skeletal muscle compared with the control group. In contrast, pyruvate levels in plasma, liver and skeletal muscle was markedly lower than normal in diabetic and starved rats. L-Lactate level lowered markedly in plasma, liver, and skeletal muscle of starved rats and elevated in liver of diabetic rats. Differences between plasma L-lactate level for diabetes and control were not significant. MG level was significantly elevated in plasma and depressed in livers and muscles of starved rats as well as livers of diabetic rats. Hepatic glycerol content was markedly increased in those states. Enzyme activities related to D- and L-lactate, such as pyruvate kinase, phosphofructokinase, aldolase and glyoxalase I, were measured in the livers of these rats. Pyruvate kinase activity decreased in these states, but other enzyme activities showed no significant changes. D-Lactate was much more excreted than L-lactate in the urine of diabetic and fasted rats compared with normal rats [81].

Wahba et al in 1992 estimated the activities of choline acetyltransferase and acetyl-cholinesterase in the seminal vesicle and in urinary bladder in streptozotocin induced diabetic male Sprague-Dawley rats. Diabetic rats exhibited significant increase in both the enzymes

compared to control animals in the detrusor muscles of urinary bladder. Significant increase in Choline acetyl transferase activity was observed only in the seminal vesicles [82].

Shibib et al in 1993 showed that Coccinia indica and Momordica charantia extracts lowered blood glucose by depressing its synthesis, on the one hand through depression of the key gluconeogenic enzymes glucose-6-phosphatase and fructose 1, 6, bisphosphatase and on the other by enhancing glucose oxidation by the shunt pathway through activation of its principal enzyme glucose-6-phosphate dehydrogenase in streptozotocin induced hyperglycaemic rats [83].

Levels of lipid peroxidation in liver, kidney, brain and blood, liver glutathione (GSH) and several enzymes in liver tissue associated with antioxidant defence mechanism, namely Catalase (EC: 1.11.1.6), GSH reductase (EC:1.6.4.2) and GSH-S-transferase (EC: 2.5.1.18), were investigated in streptozotocin-induced diabetic rats by Mukherjee et al in 1994. The single intraperitoneal injection of streptozotocin (65 mgm/kg) caused a four-, eight- and seven-fold increase in lipid peroxidation in brain, liver and kidney, respectively. A decline in GSH levels both in blood (two-fold) and liver (16%) compared with normal counterparts was also observed. A marginal increase in catalase activity, a 20% decrease in GSH reductase and an increase of GSH-S-transferase activity was also found in this experimental diabetic condition. These results suggest experimental diabetes, induced by streptozotocin, can produce biochemical changes not only in pancreas but also in liver, kidney and brain tissue [84].

Plasma levels of fibrinogen, alpha 1-acid glycoprotein (AG) and albumin, pancreatic insulitis quantitative scores, and erythrocyte velocity in the mesoappendix microvessels were measured by Guillot et al in 1994 in BB diabetic (BBD) and streptozotocin-diabetic rats (WSTZ) in order to answer the following questions : (a) Does hyperfibrinogenemia or increase in AG plasma level occur in BBD and WSTZ rats, and if so, are these alterations secondary to the hyperglycemia or to an inflammatory process such as insulitis? (b) Is there a decrease in microcirculatory flow in the BBD and WSTZ rats, and if so, is it secondary to the hyperfibrinogenemia and/or the hyperglycemia? Insulitis was present in the BBD rats after 5 weeks of disease (with a score of 2.9 +/- 0.1 vs. 1.4 +/- 0.6 in the normoglycemic controls), but absent in WSTZ rats after 5 months of disease (1.2 +/- 0.06 vs. 1.1 +/- 0.06). Increase in fibrinogen and AG plasma levels was observed in the BBD rats only and appears linked to the insulitis. The major acute phase protein AG level is increased in BBD rats already on the first day of appearance of glycosuria. In the WSTZ rats, without insulitis, chronic hyperglycemia alone did not induce an increase in fibrinogen and AG plasma levels. A decreased microcirculatory erythrocyte velocity has been found in both BBD and WSTZ rats. Thus an increase in fibrinogen

or AG plasma levels is not necessary for inducing a decrease in erythrocyte velocity. Hyperglycemia is probably the main factor responsible for the decrease in microcirculatory flow in the BBD and WSTZ rats [85].

This study by Rasschaert et al in 1994 aimed to compare the metabolic and secretory responses of pancreatic islets from animals with non-insulin dependent diabetic to D-glucose with the effects of the methyl esters of succinic acid and glutamic acid. In contrast unaltered activities of glutamate dehydrogenase and succinate dehydrogenase in the islets of diabetic animals were found [86].

Liu et al studied in 1994 the effect of acute, streptozotocin induced diabetes on hepatic microsomal glucose-6-phosphatase activity and m-RNA expression in young, juvenile and adult ratio. In control rats the m-RNA expression and enzyme activity was similar among the three age groups. The enzyme activity was increased in the streptozotocin induced diabetic rats in all groups. Glucose-6-phosphatase m-RNA expression was increased in the diabetic rats as well. So they concluded that acute streptozotocin- diabetes increase expression of glucose-6-phosphatase m-RNA and thus contributes to the increased glucose-6-phosphatase activity seen with diabetes mellitus [87].

The monomethyl ester of succinic acid (SME) was proposed as a novel tool for stimulation of proinsulin biosynthesis and insulin release in animal models of non-insulin-dependent diabetes mellitus. In this study by Giroix et al in 1994, either saline or SME (14 m mol/day) was infused for 3 days to control rats, animals injected with streptozotocin during the neonatal period and Goto-Kakizaki rats with inherited diabetes. The infusion of SME failed to correct the anomalies found in the islets of diabetic rats. These findings raise the question of whether a more prolonged administration of SME is required to raise the insulin store and improve the secretory potential of the endocrine pancreas in animals with type 2 diabetes [88].

Prickarto et al in 1995 studied the effects of chronic treatment with Acetyl-L-Carnitine on spatial discrimination learning and choline acetyl transferase activity of middle aged streptozotocin treated rats. Chronic treatment with Acetyl-L-Carnitine attenuated both the STREP induced impairment in spatial bias and the decrease in hippocampal choline acetyl transferase activity [89].

Glucose production and utilization and activities of key enzymes involved in liver and muscle glucose metabolism were studied by Burcelin et al 1995 in post absorptive streptozotocin diabetic rats after 12 hour of severe hyperglycaemia and insulinopenia. Basal glucose production

was increased; liver glycogen concentration was decreased and liver glucose-6-phosphatase activities were increased [90].

Kakkar et al in 1995 hypothesized that oxygen free radicals (OFRs) may be involved in pathogenesis of diabetic complications. They therefore investigated the levels of lipid peroxidation by measuring thiobarbituric acid reactive substances (TBARS) and activity of antioxidant enzymes [superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT)] in tissues and blood of streptozotocin(STZ)-induced diabetic rats. The animals were divided into two groups: control and diabetic. After 10 weeks (wks) of diabetes the animals were sacrificed and liver, heart, pancreas, kidney and blood were collected for measurement of various biochemical parameters. Diabetes was associated with a significant increase in TBARS in pancreas, heart and blood. The activity of CAT increased in liver, heart and blood but decreased in kidney. GSH-Px activity increased in pancreas and kidney while SOD activity increased in liver, heart and pancreas. The findings suggest that oxidative stress occurs in diabetic state and that oxidative damage to tissues may be a contributory factor in complications associated with diabetes [91].

Tormo et al in 1995 observed that intestine has a high glycolytic activity but its metabolic role could be altered in diabetes mellitus. They supplied glucose to the intestine only by the vascular route and investigated in vivo the glucose retained and the lactate produced by the intestine of normal and diabetic rats and in vitro the effect of different arterial glucose concentrations on glucose utilization and lactate, alanine and pyruvate production in normal and diabetic rats. The lactate produced was significantly higher in diabetic than in normal rats [92].

Reddy et al studied in 1995 the status of glycogen and pyruvate and the activity of glucose-6-phosphatase and alanine amino transferase in liver under the influence of sepia shell extract in both normal and streptozotocin induced diabetic mice. The glycogen concentration was elevated steeply in both and the pyruvate concentration increased substantially in diabetic mice, while the activity of glucose-6-phosphatase and alanine amino transferase was inhibited in normal and diabetic mice [93].

Bitter melon (momordica charantia) commonly known as karela, has been reported to have hypoglycaemic, antiviral, antidiabetic and anti tumour activities. Raza et al in 1996 investigated the effects of oral feeding of karela fruit juice on the hepatic cytochrome P 450 and glutathione-s-transferase drug metabolising enzymes in the streptozotocin induced diabetic rat. The cytosolic glutathione concentration was decreased in diabetic rats, and karela juice

feeding normalised the effect. However, an increase of 20-30% in the glutathione-s-transferase activity was observed in both diabetic and karela juice fed rats. In addition they also observed that karela does not always reverse the effects on drug-metabolising enzymes in STZ-induced diabetes [94].

Yao et al in 1997 showed that controversial reports on the efficacy and possible toxicity of vanadium obtained from various studies may be attributed to differences in the method of diabetes induction and (or) to differences in animal strains. The objective of this study was to evaluate the contribution of these two factors to the effects of vanadium in the treatment of experimental diabetes. Two methods of streptozotocin induction of diabetes in rats have been used for studying the antidiabetic effects of vanadium. One involves a single intravenous injection of 60 mg/kg streptozotocin, and the other uses two subcutaneous injections of 40 mg/kg streptozotocin, to either Wistar or Sprague-Dawley rats. In a 7-week chronic study, Sprague-Dawley rats appeared to develop a more severe diabetes (indicated by higher plasma cholesterol and higher fasting plasma glucose levels) following the single intravenous injection of streptozotocin than rats made diabetic by two subcutaneous injections of streptozotocin. Irrespective of the method of diabetes induction, the responses of all the diabetic animals to chronic vanadyl sulphate treatment were similar. In an acute study, Wistar diabetic rats were more responsive than Sprague-Dawley diabetic rats to vanadyl sulphate and to lower doses (0.6 and 0.8 mmol/kg) of a new organic vanadium compound, bis(maltolato)oxovanadium (i.v.) [95].

Tatsuki et al in 1997 studied the relationship between changes in lipid peroxides and those in catalase activity in pancreases, livers and hearts of streptozotocin-induced diabetic rats. Animals were killed 2 or 7 weeks after saline or streptozotocin (32 mgm/kg, i.v.) injection. The levels of blood glucose and plasma insulin in the 2-week streptozotocin-treated rats were 176.8+/-20.5 mg/dl and 29.9+/-3.2 microU/ml, respectively. In the pancreas, the lipid peroxide level significantly decreased and the catalase activity significantly increased 2 weeks after streptozotocin injection. These changes recovered after 7 weeks. In the heart, the lipid peroxide level significantly increased without any change of catalase activity 2 weeks after the initiation of diabetes. After 7 weeks, the catalase activity significantly increased and the lipid peroxide level returned to the control level. In the liver, there was no change in the lipid peroxides and catalase in the 2-week streptozotocin-treated rats, whereas the catalase activity significantly increased 7 weeks after the injection. It was suggested that the defense system in the pancreas to oxidative stress may be evoked in an early stage of streptozotocin-induced diabetes [96].

It is known that streptozotocin, penetrating into the organism generates nitrogen monoxide (NO). Therefore, it is justified to presume that in beta-cell destruction thereby induced, peroxinitrite resulting from NO and superoxide (O_2^-) reaction has an important role. Matkovics et al in 1997 tested and compared antioxidant enzymes (superoxide dismutase, glutathione peroxidase and catalase activities) glutathione reductase activity regenerate reduced glutathione. The oxidized, reduced glutathione values and lipid peroxidation changes were measured. From the studies, it has appeared that streptozotocin treatment generally induces an oxidative predominance in tissues [97].

Oxygen free radicals have been suggested to be a contributory factor in complications of diabetes mellitus. There are many reports indicating the changes in parameters of oxidative stress in diabetes mellitus. In this study Kakkar et al in 1998 aimed to identify whether oxidative stress occurs in the liver and pancreas in the initial stages of development of diabetes. They therefore investigated the lipid peroxide level (thiobarbituric acid-reactive substances, TBARS) and activities of antioxidant enzymes [superoxide dismutase (SOD), catalase and glutathione peroxidase] in liver and pancreas of control and streptozotocin-induced diabetic rats at various stages of development of diabetes. Male Sprague-Dawley rats were divided into two groups: group I, control (n = 42) and group II, diabetic (n = 42). Each group was further subdivided into seven groups consisting of six rats each. Rats in these subgroups were studied at weekly intervals (0 to 6 weeks). Plasma glucose levels, TBARS levels and activities of antioxidant enzymes were measured in liver and pancreas at various time intervals. There was a significant ($P < 0.05$) and progressive increase in TBARS levels of liver and pancreas in the diabetic group. Total SOD and Cu-Zn-SOD activity increased ($P < 0.05$) with progression of diabetes while Mn-SOD activity showed no significant change in either tissue. Catalase and glutathione peroxidase activities increased significantly ($P < 0.05$) in liver and pancreas. Immunohistochemical study of pancreatic islet revealed a decrease in the expression of insulin with progression of diabetes. However, glucagon and somatostatin showed an increase in immunoreactivity and a difference in their distribution pattern. The findings of the present study suggest that oxidative stress starts at early onset of diabetes mellitus and increases progressively. In conclusion, the structural damage to these tissues or complications of diabetes mellitus may be due to oxidative stress [98].

The glycogen concentration in liver is altered in various pathophysiologic states. In fasted rats, it is higher in diabetic, and lower in adrenalectomized rats compared to control animals. In fed rats, it is lower in diabetic, and little changed in adrenalectomized animals compared to

controls. Gannon et al in 1998 determined whether the activity of glycogenin, a self-glycosylating protein that initiates the synthesis of new glycogen molecules, could explain these differences in liver glycogen concentration. Glycogenin activity was measured by the incorporation of 14C-glucose from UDP-U-14C-glucose into an acid-precipitable product before and after amylase treatment of liver extracts. The glycogenin activity was similar in normal, diabetic and adrenalectomized fasted animals, regardless of the hepatic glycogen concentration. In fasted rats, glycogenin was present predominantly as the free-form of the enzyme, i.e., not attached to an amylase-digestible glycan, presumably glycogen. In contrast, in fed rats, the majority, if not all of the glycogenin was incorporated into a glycogen-like (proteoglycan) molecule. Proteoglycan synthase activity, previously identified in normal fed rats, also was present in diabetic and adrenalectomized fed rats, and the activity was similar. Thus, the altered ability to store hepatic glycogen in diabetic fed and fasted and adrenalectomized fasted rats cannot be explained by decreases in glycogenin or proteoglycan synthase activities, at least as measured using the present assays [99].

Poucheret et al demonstrated in 1985 that vanadium administered in the drinking water to streptozotocin (STZ) diabetic rats restored elevated blood glucose to normal. Subsequent studies have shown that vanadyl sulfate can lower elevated blood glucose, cholesterol and triglycerides in a variety of diabetic models including the STZ diabetic rat, the Zucker fatty rat and the Zucker diabetic fatty rat. Long-term studies of up to one year did not show toxicity in control or STZ rats administered vanadyl sulfate in doses that lowered elevated blood glucose. In the BB diabetic rat, a model of insulin-dependent diabetes, vanadyl sulfate lowered the insulin requirement by up to 75%. Vanadyl sulfate is effective orally when administered by either single dose or chronic doses. It is also effective by the intraperitoneal route. Authors have also been able to demonstrate marked long-term effects of vanadyl sulfate in diabetic animals following treatment and withdrawal of vanadyl sulfate. Because vanadyl sulfate is not well absorbed they have synthesized and tested a number of organic vanadium compounds. One of these, bismaltolato-oxovanadium IV (BMOV), has shown promise as a therapeutic agent. BMOV is 2-3x more potent than vanadyl sulfate and has shown less toxicity. Recent studies from their laboratory have shown that the effects of vanadium are not due to a decrease in food intake and that while vanadium is deposited in bone it does not appear to affect bone strength or architecture. The mechanism of action of vanadium is currently under investigation. Several studies indicate that vanadium is a phosphatase inhibitor and that vanadium can activate serine/threonine kinases distal to the insulin receptor presumably by preventing dephosphorylation

due to inhibition of phosphatases Short-term clinical trials using inorganic vanadium compounds in diabetic patients have been promising [100].

To better define the modifications of liver gluconeogenesis and citric acid cycle, or Krebs' cycle, activity induced by insulin deficiency and the effects of metformin on these abnormalities, Large and Beylot in 1999 infused livers isolated from postabsorptive or starved normal and streptozotocin-induced diabetic rats with pyruvate and lactate (labeled with [3-13C]lactate) with or without the simultaneous infusion of metformin. Lactate and pyruvate uptake and glucose production were calculated. The 13C-labeling pattern of liver glutamate was used to calculate, according to Magnusson's model, the relative fluxes through Krebs' cycle and gluconeogenesis. These relative fluxes were converted into absolute values using substrate balances. In normal rats, starvation increased gluconeogenesis, the flux through pyruvate carboxylase-phosphoenolpyruvate carboxykinase (PC-PEPCK), and the ratio of PC to pyruvate dehydrogenase (PDH) flux ($P < 0.05$); metformin induced only a moderate decrease in the PC:PDH ratio. Livers from postabsorptive diabetic rats had increased lactate and pyruvate uptakes ($P < 0.05$); their metabolic fluxes resembled those of starved control livers, with increased gluconeogenesis and flux through PC-PEPCK. Starvation induced no further modifications in the diabetic group. Metformin decreased glucose output from the liver of starved diabetic rats ($P < 0.05$). The flux through PC-PEPCK and also pyruvate kinase were decreased ($P < 0.05$) by metformin in both groups of diabetic rats. In conclusion, insulin deficiency increased in this model of diabetes gluconeogenesis through enhanced uptake of substrate and increased flux through PC-PEPCK; metformin decreased glucose production by reducing the flux through PC-PEPCK [101].

Antioxidant enzymes in liver and small intestine were investigated by Giron et al in 1999 using control and streptozotocin diabetic rats fed diets with 5% olive, sunflower or fish oil for five weeks. In liver, glutathione peroxidase and superoxide dismutase decreased and in intestine glutathione-s-transferase (GST) increased by diabetes. In isolated jejunum and ileum, this increase in GST activity was due to an increase in GST-alpha and mu isoenzymes in jejunum and GST-alpha, mu and pi in ileum. Since GST plays an important role in protecting tissues from oxidative damage, the results highlight the role of the intestine against free radicals in physiological or pathological situations [102].

The aim of this study by Kinalski et al in 2000 was to evaluate lipid peroxidation and scavenging enzyme activity in streptozotocin-induced diabetes, and then to establish whether moderate doses of nonenzymatic antioxidant vitamin E play a role in the antioxidant defence

system in diabetic pregnant rats and their offspring. The study group consisted of 30 normal female Wistar rats, which were given a single dose of streptozotocin (40 mgm/kg) and were mated 7 days later. Subsequently, the diabetic animals were divided into two matched groups: the first supplemented with vitamin E (30 mgm/100 g chow), and the other fed with a standard diet lacking vitamin E. Controls consisted of 15 pregnant rats. On the first day after delivery, the rats were decapitated and homogenates of maternal liver and uterus as well as neonatal lungs and liver were prepared. Then the following parameters were measured: malondialdehyde (MDA) concentrations in the homogenates and blood serum, glutathione (GSH) levels, the activity of CuZn-superoxide dismutase (SOD) and glutathione peroxidase (GPx), and glycaemia. The neonates of diabetic rats were smaller than the healthy ones and serum glucose concentration was markedly higher in the diabetic animals. MDA levels were significantly increased, whereas GSH, SOD and GPx were markedly diminished in the diabetic adult rats and their offspring in comparison to the control group. In the animals supplemented with alpha-tocopherol, MDA concentrations were significantly lower, GSH content and SOD activities were markedly elevated most tissues studied, whereas GPx remained unchanged. By monitoring the activity of selected scavenging enzymes, information on ongoing biological oxidative stress and thereby on the fetus/neonate status may be obtained. The results suggest that diabetic pregnant rats and their neonates are exposed to an increased oxidative stress and that vitamin E supplementation may reduce its detrimental effects [103].

Compounds of the trace element vanadium have been shown to mimic insulin in in vitro and in vivo systems. These compounds have been found to exert anti-diabetic effects in rodent models of type 1 and type 2 diabetes mellitus as well as in a limited number of studies in human diabetic subjects. Thus, vanadium compounds have emerged as agents for potential use in diabetes therapy. However, treatment of diabetic animals with inorganic vanadium salts has also been associated with some toxic side-effects such as gastrointestinal discomfort and decreased body weight gain. In addition, vanadium salts have been reported to exert toxic effects on the liver and kidney. More recently, it was shown that organic vanadium compounds were much safer than inorganic vanadium salts and did not cause any gastrointestinal discomfort, hepatic or renal toxicity. This review by Srivastava in 2000 briefly summarizes the anti-diabetic and toxic effects of vanadium compounds [104].

Clore et al in 2000 determined rates of endogenous glucose production and the activities of glucose-6-phosphatase and glucokinase in obese patients scheduled for gastric by pass surgery. Hepatic glucose-6-phosphatase activity determined from freshly isolated microsomes

was significantly increased in the type 2 diabetic patients compared with the obese control subjects [105].

In streptozotocin (STZ)-induced diabetic rats, the authors previously showed an increased heparin-releasable (luminal) lipoprotein lipase (LPL) activity from perfused hearts. To study the effect of this enlarged LPL pool on triglyceride (TG)-rich lipoproteins, Sambandam et al in 2000 examined the metabolism of very-low-density lipoprotein (VLDL) perfused through control and diabetic hearts. Diabetic rats had elevated TG levels compared with control. However, fasting for 16 h abolished this difference. When the plasma lipoprotein fraction of density <1.006 g/ml from fasted control and diabetic rats was incubated in vitro with purified bovine or rat LPL, VLDL from diabetic animals was hydrolyzed as proficiently as VLDL from control animals. Post-heparin plasma lipolytic activity was comparable in control and diabetic animals. However, perfusion of control and diabetic rats with heparinase indicated that diabetic hearts had larger amounts of LPL bound to heparan sulfate proteoglycan-binding sites. [(3)H]VLDL obtained from control rats, when recirculated through the isolated heart, disappeared at a significantly faster rate from diabetic than from control rat hearts. This increased VLDL-TG hydrolysis was essentially abolished by prior perfusion of the diabetic heart with heparin, implicating LPL in this process. These findings suggest that the enlarged LPL pool in the diabetic heart is present at a functionally relevant location (at the capillary lumen) and is capable of hydrolyzing VLDL. This could increase the delivery of free fatty acid to the heart, and the resultant metabolic changes could induce the subsequent cardiomyopathy that is observed in the chronic diabetic rat [106].

It has recently been shown that food intake is not essential for the resynthesis of the stores of muscle glycogen in fasted animals recovering from high-intensity exercise. Because the effect of diabetes on this process has never been examined before, Ferreira et al in 2001 undertook to explore this issue. To this end, groups of rats were treated with streptozotocin (60 mgm/kg body mass ip) to induce mild diabetes. After 11 days, each animal was fasted for 24 h before swimming with a lead weight equivalent to 9% body mass attached to the tail. After exercise, the rate and the extent of glycogen repletion in muscles were not affected by diabetes, irrespective of muscle fiber composition. Consistent with these findings, the effect of exercise on the phosphorylation state of glycogen synthase in muscles was only minimally affected by diabetes. In contrast to its effects on nondiabetic animals, exercise in fasted diabetic rats was accompanied by a marked fall in hepatic glycogen levels, which, surprisingly, increased to preexercise levels during recovery despite the absence of food intake [107].

Streptozotocin (STZ), an analogue of GlcNAc, inhibits purified rat spleen O-GlcNAc-selective N-acetyl-beta-D-glucosaminidase (O-GlcNAcase), the enzyme that removes O-GlcNAc from protein. STZ increases pancreatic islet O-linked protein glycosylation. In light of these data, Konrad et al in 2001 investigated the possibility further that STZ causes beta-cell death by inhibiting O-GlcNAcase. In isolated islets, the time course and dose curve of STZ-induced O-glycosylation correlated with beta-cell toxicity. STZ inhibition of rat islet O-GlcNAcase activity also paralleled that of its beta-cell toxicity, with significant inhibition occurring at a concentration of 1 mM. In contrast, STZ inhibition of rat brain O-GlcNAcase and beta-TC3 insulinoma cell O-GlcNAcase was significantly right-shifted compared with islets, with STZ only significantly inhibiting activity at a concentration of 5 mM, the same concentration required for beta-TC3 cell toxicity. In comparison, N-methyl-N-nitrosourea, the nitric oxide-donating portion of STZ, did not cause increased islet O-glycosylation, beta-cell toxicity or inhibition of beta-cell O-GlcNAcase. Enhanced STZ sensitivity of islet O-GlcNAcase compared with O-GlcNAcase from other tissues or an insulinoma cell line suggests why actual islet beta-cells are particularly sensitive to STZ. Confirming this idea, STZ-induced islet beta-cell toxicity was completely blocked by GlcNAc, which also prevented STZ-induced O-GlcNAcase inhibition, but was not even partially blocked by glucose, glucosamine or GalNAc. Together, these data demonstrate that STZ's inhibition of beta-cell O-GlcNAcase is the mechanism that accounts for its diabetogenic toxicity [108].

2.2 Role of Selenium on Diabetes :

Accepted methods of treatment of Diabetes Mellitus in patients vary in their effects in producing normoglycaemia. Normoglycaemia or near normal blood sugar level is difficult to attain with any of the antidiabetic regimes and is even more difficult to maintain over a period of time. Moreover, antidiabetic regimes are associated with considerable possible side effects and they require strict clinical monitoring, imparting a burden of considerable cost, restriction in life style and training on patient's part. Maintenance of normoglycaemia over a period of time is, however, nodal in management of diabetes and prevention of diabetic complication. It is mandatory to maintain normoglycaemia in certain conditions like pregnant diabetic and diabetes with renal transplantation. Regimes to maintain normoglycaemic level strictly over a period of time are costly, cumbrous and restrictive.

It is clear from above discussion that search of a relatively cheap, non-toxic, less restrictive and inexpensive management regime of diabetes other than the accepted one, is a matter of great practical interest. Study of selenium intake in experimentally produced diabetic mice as a model, gives us important insight in problems of diabetic management and metabolism. A gamut of previous work discussed below entails the effect of selenium in diabetic mice.

Slonim et al in 1983 studied the effect of antioxidant vitamin E, administered prior to administration of diabetogenic agents, in mice. This was done in addition to study of diabetic susceptibility in mice, fed on vit. E and selenium deficient diet. It was observed that the mice, fed on vit E, Selenium deficient diet, showed increased susceptibility to diabetes development by ordinarily non-diabetogenic doses of streptozotocin. It pointed out that selenium in diet might have a role in prevention of diabetes in animal model. In our study we got results corroborated with the said hypothesis more so because our study examined about reversal of diabetic state in diabetic mice by selenium in diet [109].

Dohi et al in 1988 studied the activity of tissue glutathione peroxidase, a selenium dependent enzyme, in rats 4 & 8 months after injection of streptozotocin. Some other enzymes like catalase and superoxide dismutase were also studied. Selenium dependent glutathione peroxidase activity was increased in kidney, unaltered in lung & liver and significantly decreased in aorta. Catalase activity was uninfluenced by diabetic state in rat aorta. Superoxide dismutase activity was less than detectable. Catalase and superoxide dismutase activity was unaltered in kidney in diabetic state. The observed anomalies in this study are corrected by reversal of diabetic state by insulin treatment in animal model. It is depicted from the study that repletion of selenium dependent

glutathione peroxidase in rat aorta is dependent on reversal of diabetic state and hence are interrelated. In part of the study they studied the effect of selenium feeding in diabetic mice on liver catalase activity and found no significant alteration [110].

Flechner et al in 1990 studied the effect of radical scavengers in the prevention of β cell destruction by streptozotocin in animal model of BB rat. Eight compounds were tested either in isolation or in combination. No effect was seen on diabetic state for 3 aminobenzamide, N acetyl DL homocysteine thiolactone, ebselen and butylated hydroxyanisole whereas partial suppression of hyperglycaemia was seen with cysteamine. In BB rats diabetes development was delayed and hyperglycaemia partially suppressed by administration of ebselen and vit E. and Max EPA (fish lipid concentrate). Authors conclude that diabetic state was not readily modifiable by exogenous radical scavengers. In the study, however, intervention with oral selenium interfered significantly with development of diabetic state in animal model [111].

Mc Neill et al in 1991 found that the treatment of streptozocin (STZ)-induced diabetic rats with sodium selenate (10-15 mumol.kg-1.day-1) for 7 wk resulted in a decrease in plasma glucose, food intake, and water intake to control or near control levels. Plasma insulin was reduced in control rats given sodium selenate to the level found in the diabetic and treated diabetic group. Treatment did not affect control rats with regard to the other measurements cited. Sodium selenate enhanced weight gain in responding diabetic rats to that seen in controls; sodium selenate's actions thus resembled those of insulin. Thus selenate, like vanadium, appears to have insulinlike effects when administered in vivo [112].

A study in 1992 showed more valuable insight in interaction between selenium & development of diabetic state in animal model (Mukherjee et al 1992). It showed considerable beneficial effect in reduction of hyperglycaemia in streptozotocin induced diabetic mice. Blood glucose level and uptake of selenium in pancreas were monitored and matched with normal controls. Selenium uptake in pancreas was increased in diabetic mice in relation to control as well as hyperglycaemia was suppressed [113].

In an impressive work Iizuka et al in 1992, studied the effect of selenium on blood sugar and insulin levels in different varieties of diabetic rats, one induced by streptozotocin and the other by pancreatectomy. Direct action of selenium on islet tissues of diabetic rat was also studied. Drastic and fast reduction of very high blood sugar level in acute diabetic state was shown to occur by single parenteral injection of selenium as well as increase in serum insulin. However, rats with chronic diabetic state showed a normoglycaemic effect on parenteral injection

of selenium for 4 days without any significant increase in insulin levels. Pancreatectomised diabetic rats show a tendency of reduction of blood sugar level without any increase in insulin level. Selenium was shown to increase insulin release from islets in a dose dependent manner. This study showed important potential of selenium as a blood glucose lowering agent in animal model in different diabetic states as well as throws light on the mechanism of such action through pancreas by release of insulin or on effect independent of release of insulin [114].

Ghosh et al in 1994 studied the effect of oral selenium on setreptozotocin induced diabetic mice. Hyperglycaemia, decrease in hepatic glycogen, increase in glucose 6 phosphatase activity and significant decrease in plasma insulin levels and protein kinase activity are studied as established parameters in diabetic mice. Reversal to near normal values is observed in diabetic mice on oral selenium but no effect on control sample. It shows the potential of selenium as an intervention agent in diabetic with little chance to overcorrect the parameters and development of side effects like hypoglycaemia [115].

Insulin is known to be capable of regulating cellular and metabolic processes through action on gene expression and subsequent effect on enzyme activity. This knowledge gives rise to use insulin mimetic agents in experimental model in studying the effect of activity on different enzymes, to gain insight into mechanism of action of insulin on metabolism as a whole as well as on the specific enzyme activity. Berg et al made an important work in 1995 in which they used Vanadate and Selenate, known to control blood sugar level in experimental diabetic model in vivo (an insulin mimetic action). Though Vanadate is known to influence expression of several enzymes in vitro and in vivo, studies about Selenate have not been reported. The authors showed that administration of Vanadate or Selenate in streptozotocin induced diabetic rats restore activity of glucose-6-phosphate dehydrogenase and fatty acid synthetase in tissues in addition to normalisation of blood sugar levels. A normal control and a streptozotocin induced diabetic rat, treated with insulin, are also studied. Comparison of activity of G6PD and FAS activity are markedly reduced in streptozotocin induced diabetic rats than in normal control with restoration of activity by insulin or Vanadate/Selenate administration upto 80-90% of normal level with no significant difference in result from insulin and Vanadate/Selenate group in between increase in G6PD or FAS are due to increase in cellular mRNA level. Increase in cellular mRNA, responsible for increased synthesis of G6PD & FAS, suggests the effect of insulin or mimetics (Vanadate/Selenate in this case) occurs pretranslationally through gene regulation [116].

Saito et al in 1995 found that tissue selenium was decreased in streptozotocin treated old rats and this decrement was probably related to the peroxidation in tissue damage [117].

Further evidence of insulin mimetic action of selenium independent of insulin was obtained from a breakthrough work of Becker et al in 1996. On feeding with sodium selenate (Na_2SeO_4) for 10 weeks, streptozotocin induced insulin deficient diabetic rats show amelioration of hyperglycaemia (appx. 25 mmol) and glucosuria (appx. 85 mmol/day) by 50% and 80% respectively when compared to a similar untreated diabetic control group. Comparison of oral and intravenous GTT (glucose tolerance test) shows reduction of 40-50% in blood glucose levels after a glucose load when compared to untreated control. These activities were not accompanied by a rise in plasma insulin levels. In fact insulin reserves are reduced more than 90% by streptozotocin in treated and untreated diabetic rats. The hepatic activity of two key glucolytic enzymes, glucose and L pyruvate kinase as well as corresponding mRNA activity were increased approximately two-three fold to reach 40-75% of normal. In diabetic rats activity of these two enzymes were blunted. In addition gluconeogenic enzyme phosphoenolpyruvate carboxykinase, elevated in streptozotocin induced diabetic rats, were reduced by selenate treatment with parallel changes in correspondent mRNA. Selenium, an anorexigenic, induces reduction of body weight up to a moderate extent, a calorie restricted weight matched similar control of diabetic rats were run and show no improvement in glucose homeostasis and enzyme effects compared to selenate group. In addition no obvious untoward effect on kidney or liver was observed for selenium treatment in diabetic rats. The study showed selenium improved glucose homeostasis and diabetic state in animal model through an insulin like action but independent of insulin by correction of pretranslational regulatory mechanism in hepatic metabolism of glucose [118].

Douillet in 1996 studied the effect of selenium and selenium supplemented with Vit. E on kidney in diabetic rats. Some protection against development of pathology in reduction of renal hyperfiltration and dissemination in number and severity of diabetic glomerular lesion is evidenced [119].

A study of selenium supplement (selenomethionine) along with Vit. E on platelet activity in diabetic rats in vitro was shown by Douillet in 1996 to effect a reduction in platelet thrombin, ADP induced aggregation, in adhesiveness to fibronectin and in sorbitol content. Platelet selenium is significantly increased alongwith prevention of diabetic oxidative damage to platelet membrane [120].

Fifty-two healthy Swiss Male Albino rats aged two mo were used in the study by Gumuslu et al in 1997 . They were divided into four groups: control (C), diabetic (D), cadmium (Cd), and diabetic + Cd (D + Cd) groups. Diabetic condition was induced in D and D + Cd groups

by administration of alloxane (5 mg/100 g). After this treatment, Cd and D + Cd groups were injected with CdCl2 i.p. (2 mg/kg/wk). At the end of the 2-mo experimental period, thiobarbituric acid reactive substances (TBARS), plasma and erythrocyte selenium (Se), plasma ceruloplasmin (Cp), and vitamin E (vit E) were determined in four groups of rats. The erythrocyte Se was lower in the experimental groups than in the controls. Plasma Se was significantly decreased in the D and D + Cd groups compared with the control group. Plasma Cp was unaltered. Plasma vit E was significantly decreased in Cd group in comparison with the C, D, and D+Cd groups [121].

Battell in 1998 substantiated again the event of normoglycaemia in streptozotocin induced diabetic rat by selenium independent of insulin release. At 8 weeks of diabetes selenium treated diabetic group are found to have normal heart functions in comparison to untreated diabetic group [122].

Benefical effects of seleniomethionine and Vit. E supplements were impresively demonstrated in tissues like liver, kidney, heart and aorta of diabetic rat after 24 weeks of induction by streptozotocin in a study by Douillet in 1998. Diabetes induced increase in thiobarbituric acid reactive substances, conjugated dienes and decrease in triglyceride and phospholipid levels in liver were beneficially corrected to a large extent by supplement. Decrease in 18 : 2n-6 and increase in 22 : 6n-3 observed in diabetic rat liver as a reflection of altered glycaemic control were beneficially reversed. The increase in cardiac triglyceride induced by diabetes was reversed alongwith increase in 18 : 0 ether linked alcohol, 20 : 4n-6 and 22 : 5n-3 level in cardiac lipids. In aorta 20 : 5n-3 was increased. These polyunsaturated fatty acids are precursors of PGI_2 and PGI_3 seemingly involved in cardiovascular protection. In kidney decreased 20 : 4n-6, the precursor of thromboxane A_2 by selenium supplements confers nephroprotection as thromboxane A_2 is implicated conversely to glomerular injury. Thus selenium or selenium and Vit E play a role in controlling oxidative status and altered lipid metabolism in liver, producing favourable fatty acid distribution in major tissues, affected by diabetic complication [123].

Interrelation of blood glucose, lipid peroxidation, glutathione, glutathione peroxidase and glutathione S transferase activity and blood selenium levels in streptozotocin induced diabetic mice, treated with selenium is studied by Mukherjee et al in 1998 to gain important insight in beneficial activity of selenium and the mechanism of action. Diabetes induced hyperglycaemia (2.8 fold increase), increase in malondialdehyde levels (89% in liver and 83% in blood), marked decrease in glutathione (approximately 73% in blood and 79% in liver), increase in glutathione

S transferase activity (55%) after 5 weeks of streptozotocin treatment, were reverted to normal by sodium selenite supplement. It is suggestive of a major role of selenium in reducing oxidative stress associated with diabetes [124].

Metabolic abnormalities observed in retina and in cerebral cortex were compared in diabetic rats and experimentally galactosemic rats by Kowluru et al in 1999. Diabetes or experimental galactosemia of 2 months duration significantly increased oxidative stress in retina, as shown by elevation of retinal thiobarbituric acid reactive substances (TBARS) and subnormal activities of antioxidant defense enzymes, but had no such effect in the cerebral cortex. Activities of sodium potassium adenosine triphosphatase [(Na, K)-ATPase] and calcium ATPase became subnormal in retina as well as in cerebral cortex. In contrast, protein kinase C (PKC) activity was elevated in retina but not in cerebral cortex in the same hyperglycemic rats. Dietary supplementation with an antioxidant mixture (containing ascorbic acid, Trolox, alpha-tocopherol acetate, N-acetyl cysteine, beta-carotene, and selenium) prevented the diabetes-induced and galactosemia-induced elevation of retinal oxidative stress, the elevation of retinal PKC activity and the decrease of retinal ATPases. In cerebral cortex, administration of the antioxidant diet also prevented the diabetes-induced decreases in (Na,K)-ATPase and calcium ATPases, but had no effect on TBARS and activities of PKC and antioxidant-defense enzymes. The results indicate that retina and cerebral cortex differ distinctly in their response to elevation of tissue hexose, and that cerebral cortex is more resistant than retina to diabetes-induced oxidative stress. The greater resistance to oxidative stress in cerebral cortex, as compared to retina, is consistent with the resistance of cerebral cortex to microvascular disease in diabetes, and with a hypothesis that oxidative stress contributes to microvascular disease in diabetes. Dietary supplementation with these antioxidants offers a means to inhibit multiple hyperglycemia-induced retinal metabolic abnormalities [125].

Naziglu in 1999 showed protective effect of Vit C, Vit E and selenium against oxidative damage to lens. However, the effect of Vit C here far outweighed the effect of other two supplements. In peritoneally administered Vit C, Vit E and selenium were shown to have protective effects by estimation of lipid peroxidation, glutathione peroxidase, reduced glutathione activities in lens of diabetic rats [126].

Effects of hyperglycemia (both diabetes and experimental galactosemia) on cardiac metabolism have been determined by Kowluru et al in 2000. In addition, the effect of supplemental antioxidants on these hyperglycemia-induced abnormalities of cardiac metabolism has been investigated. Diabetes or experimental galactosemia of 2 months duration in rats

significantly increased oxidative stress in myocardium, as demonstrated by elevation of thiobarbituric acid reactive substances (TBARS) and lipid fluorescent products in left ventricle. Activity of protein kinase C (PKC) was elevated in the myocardium, and the activities of (Na,K)-ATPase and calcium ATPases were subnormal. Administration of supplemental antioxidants containing a mixture of ascorbic acid, Trolox; alpha-tocopherol acetate, N-acetyl cysteine, beta-carotene, and selenium prevented both the diabetes-induced and galactosemia-induced elevation of oxidative stress and PKC activity, and inhibited the decreases of myocardial (Na,K)-ATPase and calcium ATPases. The results show that these metabolic abnormalities are not unique to diabetes per se, but are secondary to elevated blood hexose levels, and supplemental antioxidants inhibit these metabolic abnormalities. Our findings suggest that antioxidants inhibit abnormal metabolic processes that may contribute to the development of cardiac disease in diabetes, and offer a potential clinical means to inhibit cardiac abnormalities in diabetes [127].

Goemen et al in 2000 studied the effect of vitamin E and sodium selenate treatment on the neurogenic and endothelium-dependent relaxation of isolated corpus cavernosum obtained from streptozotocin-induced diabetic mice. Relaxant responses of corpus cavernosum precontracted by phenylephrine to electrical field stimulation and to acetylcholine were significantly decreased in diabetic mice. There was no significant difference between diabetic and non-diabetic groups for the relaxant response of corpus cavernosum to sodium nitroprusside and papaverine. Treatment with sodium selenate, but not vitamin E, partially prevented the impairment of the neurogenic relaxation, whereas both had a significant, partial restorative action on endothelial dysfunction in corpus cavernosum obtained from diabetic groups. Neither agent exhibited a significant action on the relaxant responses of corpus cavernosum obtained from non-diabetic mice. A decrease in the sensitivity of the neurogenic impairment to antioxidant action may develop more rapidly than that of endothelial dysfunction in streptozotocin-induced diabetic mice [128].

Severe steroidogenic and spermatogenic alterations are reported by Unlucerci et al in 2000 in association with diabetic manifestations in humans and experimental animals. This study was planned to determine whether oxidative stress is involved in diabetes-induced alterations in the testes. Diabetes was induced in male rats by injection of 50 mg/kg of streptozotocin (STZ). Ten weeks after injection of STZ, levels of selenium and activities of selenium dependent-glutathione peroxidase (GPx) and phospholipid hydroperoxide glutathione peroxidase (PHGPx) were measured in rat testis. Lipid and protein oxidations were evaluated as measurements of testis malondialdehyde (MDA) and protein carbonyl levels, respectively. Testis sulfydryl (SH)

levels were also determined. The control levels of GPx and PHGPx activities were found to be 46.5 +/- 6.2 and 108.8 +/- 19.8 nmol GSH/mgm protein/min, respectively. Diabetes caused an increase in testis GPx (65.0 +/- 21.1) and PHGPx (155.9 +/- 43.1) activities but did not affect the levels of selenium or SH. However, the testis MDA and protein carbonyl levels as markers of lipid and protein oxidation, respectively, did not increase in the diabetic group. Aminoguanidine (AG) treatment of diabetic rats returned the testis PHGPx activity (136.5 +/- 24.9) to the control level but did not change the value of GPx activity (69.2 +/- 17.4) compared with diabetic group. MDA and protein carbonyl levels in testis were not affected by AG treatment of diabetic rats, but interestingly AG caused SH levels to increase. The results indicate that reactive oxygen radicals were not involved in possible testicular complications of diabetes because diabetes-induced activations of GPx and PHGPx provided protection against oxidative stress, which was reported to be related to some diabetic complications [129].

Naziroglu et al in 2001 determined the protective effects of intraperitoneally administered vitamin E and selenium (as Na2SeO3, Se) on the lipid peroxidation as thiobarbituric acid reactive substances (TBARS) and vitamin E levels, glutathione peroxidase (GSH-Px), reduced glutathione (GSH) activities in the plasma, red blood cell (RBC), liver, and muscle,of rats with streptozotocin-induced diabetes. Fifty adult male Wistar rats were used and all rats were randomly divided into five groups. The first group was used as a control and the second group as a diabetic control. A placebo was given to first and second groups by injection. The third group was intraperitoneally administered with vitamin E (20 mg over 24 h), the fourth group with Se (0.3 mg over 24 h), and the fifth group with vitamin E and Se combination (COM) (20 mg vitamin E + 0.3 mg Se over 24 h). This administration was done for 25 days and the TBARS, vitamin E, GSH-Px, GSH levels in the plasma, RBC, liver, and muscle samples were determined. The vitamin E level in the plasma and liver was significantly ($p < 0.05$) higher in the control than in the diabetic control group. Also, the TBARS levels in the RBC, liver and muscle were significantly ($p < 0.05$) lower in the control than in the diabetic control group. However, GSH-Px and GSH activities in RBC, liver and muscle were not statistically different between the control and the diabetic control groups. The vitamin E levels in plasma and liver ($p < 0.01$ and $p < 0.001$) and GSH-Px activities ($p < 0.01$, $p < 0.001$) in RBC were significantly higher in vitamin E, Se, and COM groups than in both control and diabetic control groups. However, the TBARS levels of RBC, muscle, and liver in vitamin E and Se administered groups were significantly ($p < 0.05$-$p < 0.001$, respectively) decreased. These results indicate that intraperitoneally administered vitamin E and Se have significant protective effects on the blood, liver, and muscle against oxidative damage of diabetes [130].

Diabetes results in various biochemical abnormalities in the retina, but which of these abnormalities are critical in the development of retinopathy is not known. Kowluru et al in 2001 examined the effect of antioxidant supplementation on diabetes-induced alterations of retinal glutamate, and explored the inter-relationship between alterations of retinal glutamate, oxidative stress, and nitric oxide (NO) in diabetes. Glutamate was measured in the retina at 2 months of diabetes in rats receiving diets supplemented with or without a mixture of antioxidants containing ascorbic acid, Trolox, DL alpha-tocopherol acetate, N-acetyl cysteine, beta-carotene and selenium. The relationship between glutamate, oxidative stress and NO was evaluated using both bovine retinal endothelial cells and normal rat retina. In diabetes, retinal glutamate was elevated by 40, thiobarbituric acid-reactive substances (TBARS) by 100, and NO by 70%, respectively. Administration of antioxidants inhibited the diabetes-induced increases in glutamate, TBARS and NO. Incubation of bovine retinal endothelial cells or normal rat retina with glutamate significantly increased TBARS and NO, and addition of either antioxidant (N-acetyl cysteine) or a NO synthase inhibitor prevented the glutamate-induced elevation in oxidative stress and NO. Incubation of retina with a glutamate agonist, likewise elevated oxidative stress and NO, and memantine inhibited such elevations. Thus, the alterations of retinal glutamate, oxidative stress and NO appear to be inter-related in diabetes and antioxidant therapy may be a suitable approach to determine the roles of these abnormalities in the development of diabetic retinopathy [131].

BACKGROUND : Oxidative stress has been implicated in the pathogenesis of diabetic nephropathy. Although glucose itself can initiate oxidative stress, deficiency of essential trace elements such as selenium (Se) may exacerbate this oxidative stress in diabetic rats. The mechanism by which Se deficiency causes oxidative stress and renal injury is not completely understood. This study by Reddi et al in 2001 tested the hypothesis that Se deficiency induces renal oxidative stress and renal injury via transforming growth factor-beta1 (TGF-beta1). METHODS: Fifty-four male Wistar rats were used. Diabetes was induced in 27 rats by streptozotocin, and the other 27 rats received buffer only. Ten weeks after induction of diabetes, both normal and diabetic rats were killed, their kidneys removed, and glomeruli were isolated. Glomeruli from normal and diabetic rats were incubated in the presence of TGF-beta1 alone or its neutralizing antibody. Antioxidant enzyme (Cu-Zn) superoxide dismutase (Cu-Zn SOD), catalase, and glutathione peroxidase (GSH-Px) activities; total glutathione; and lipid peroxidation were determined. For Se studies, 15 normal and 15 diabetic rats were divided into groups of five each and fed either a regular, Se-deficient, or Se-supplemented diet one week after induction

of diabetes. Ten weeks after feeding these diets, rats were killed and glomeruli were isolated. Oxidative stress was examined by determining the mRNA expressions for antioxidant enzymes and also for TGF-beta1. Plasma glucose and albuminuria were determined. Histology of the kidney and interlobular artery was evaluated by light microscopy. RESULTS: In vitro studies showed that TGF-beta1 significantly reduced glomerular catalase and GSH-Px activities as well as total glutathione levels with an increase in lipid peroxidation in both normal and diabetic rats. Antibody to TGF-beta abrogated these changes. There was no effect of TGF-beta1 on Cu-Zn SOD. Like TGF-beta1, a Se-deficient diet caused a significant decrease in glomerular mRNA expression for Cu-Zn SOD, catalase, and GSH-Px, but a significant increase in TGF-beta1 mRNA expression. Also, a Se-deficient diet caused an increase in albuminuria, glomerular sclerosis, and plasma glucose levels in both normal and diabetic rats. The deficient diet caused a decrease in the lumen size of the interlobular artery. Se supplementation to diabetic rats up-regulated mRNA expression for antioxidant enzymes, and significantly reduced but did not normalize that of TGF-beta1. Glomerular sclerosis was normalized and the interlobular artery lumen size was greatly enlarged in diabetic rats by Se supplementation. Also, the tubulointerstitium was preserved by Se supplementation in diabetic rats. CONCLUSIONS: The data show that TGF-beta1 is a pro-oxidant and Se deficiency increases oxidative stress via this growth factor. In addition, Se deficiency may simulate hyperglycemic conditions. Se supplementation to diabetic rats prevents not only oxidative stress but renal structural injury, as well [132].

The reference ranges of the trace elements Al, As, Be, B, Cd, Co, Cu, Fe, Mn, Mo, Ni, Pb, Li, Rb, Se, Sr, and Zn were determined by inductively coupled plasma-mass spectrometry (ICP-MS) in sera of a group of free-ranging plains viscachas of the pampa grasslands of Argentina. The values were compared with those of a small group of captive plains viscachas of the Zurich Zoo with diabetes and bilateral cataracts. In addition, a method for digestion of whole-blood samples is described for the trace element determination. Significant differences in the concentration of trace elements in the two groups of animals are discussed. No correlation was found by Forrer et al in 2001 between the levels of selenium and of other trace elements compared to the formation of cataracts [133].

Kowluru et al in 2001 administered antioxidants to diabetic rats and experimentally galactosemic rats to evaluate the ability of these agents to inhibit the development of diabetic retinopathy. Alloxan diabetic rats and nondiabetic rats that were fed 30% galactose randomly received standard diets or the diets supplemented with ascorbic acid and alpha-tocopherol (vitamins C+E diet) or a more comprehensive mixture of antioxidants (multi-antioxidant diet),

including Trolox, alpha-tocopherol, N-acetyl cysteine, ascorbic acid, beta-carotene, and selenium. Diabetes or galactose feeding of at least 12 months resulted in pericyte loss, acellular capillaries, and basement membrane thickening. Compared with diabetic controls, the development of acellular capillaries was inhibited by 50% ($P < 0.05$) in diabetic rats that received supplemental vitamins C+E, and the number of pericyte ghosts tended to be reduced. The vitamins C+E supplement had no beneficial effect in galactosemic rats, but these rats consumed only approximately half as much of the antioxidants as the diabetic rats. The multi-antioxidant diet significantly inhibited (approximately 55-65%) formation of both pericyte ghosts and acellular capillaries in diabetic rats and galactosemic rats ($P < 0.05$ vs. controls), without affecting the severity of hyperglycemia. Parameters of retinal oxidative stress, protein kinase C activity, and nitric oxides remained elevated for at least 1 year of hyperglycemia, and these abnormalities were normalized by multi-antioxidant therapy. Thus, long-term administration of antioxidants can inhibit the development of the early stages of diabetic retinopathy, and the mechanism by which this action occurs warrants further investigation. Supplementation with antioxidants can offer an achievable and inexpensive adjunct therapy to help inhibit the development of retinopathy in diabetes [134].

In order to study the metabolism of essential trace elements in diabetics, Feng et al in 2001 studied alloxan-diabetic rats for the distribution patterns of chromium (Cr), cobalt (Co), iron (Fe), selenium (Se), and zinc (Zn) in the liver, kidney, pancreas, and testes, as well as in the organ subcellular fractions. Normal rats were used as controls. Cr 50-enriched stable isotopic tracer solution was given by intravenous injection to avoid the difficulties of estimation of Cr status. Data showed that the concentrations of Zn in liver and kidney, of Co, Fe, and Zn in pancreas, and of Fe and Zn in testes of the diabetic rats were significantly higher than in the control rats. Nevertheless, the concentrations of Cr in pancreas, Fe in kidney, and Cr and Se in testes of the diabetic rats were significantly lower than in the controls. Furthermore, they observed significant alterations of element concentrations in subcellular fractions of various organs in the diabetic rats. These results suggest that changing hormone levels may interfere with the accumulation of some trace elements both in the organs and in the subcellular fractions of rats [135].

So this review shows that study of the effect of antioxidant like Vit E, selenium etc. on experimentally induced diabetes was the first line of research work started on free radical scavengers on diabetes.

This was a chance finding that selenium showed to have a normoglycaemic effect in experimentally induced hyperglycaemia.

Vanadium like selenium has an insulin like effect to lower the blood sugar level in diabetes. Studies then looked for different biochemical markers and enzymes related to different biochemical pathways in diabetes and the effects of selenium in regulating these pathways.

Gradually the researchers found interest in the complication of diabetes and started working on the beneficial effect of antioxidants on the long term complications like cataract, nephropathy, retinal metabolism, cardiomyopathy etc.

Considering these facts we studied the effects of selenium on carbohydrate, fat, protein metabolism and the action on hepatic microsomal enzymes and the peroxidative processes.