DISCUSSION

6.0 Discussion :

Diabetes has reached an alarming state and is found to be a powerful predisposing factor for many diseases in general. The identification of the independent unique effect in diabetes still remains to be a chalanging problem and have implecated the needs for continued research.

The sugar glucose is the most important carbohydrate and it is as glucose that the bulk of dietary carbohydrate is absorbed into the blood-stream or into which it is converted in the liver and it is from glucose that all other carbohydrates in the body can be formed [177]. In diabetes as glucose is not utilised in the cell the blood sugar level rises.

Chemical toxins such as streptozotocin have been shown to cause abrupt onset of diabetes in a variety of animals including rat, mouse, Chinese hamster, dog, sheep, rabbit and monkey.

Streptozotocin-2-Deoxy-2-[{(methylnitrosoamino)-carbonyl} amino]-D-glucopyranose; or 2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose.

$C_8H_{15}N_3O_7$	-	molecular weight 265.22
LD_{50} in female mice	-	360 mgm/Kg intraperitoneal 275 mgm/Kg intravenous [178].

Damaged β cells initially exhibit a vacuolated cytoplasm followed by shrinkage of the nuclei as the cells detach from one another. As cellular destruction proceeds nuclear damage and disintegration of the nuclear membrane become obvious. Generally β cell destruction is complete by 24 hours after the toxin is given. Following significant β cell loss, the islets in diabetic animals are small. The islets cells are arranged in small cords that are surrounded by fibrous tissue. Using immunocytochemical techniques, it has been demonstrated that these cords are composed of two thirds glucagon containing α-cells and one third somatostatin containing δ cells. Insulin containing β cells are almost totally absent in these islets.

There is no effective regeneration of β cells in the islets of adult animals treated with islet cell toxins; however, in neonates there is marked regenaration of β cells following a toxic insult. Neonatal rats treated with a high dose of streptozotocin show a dramatic increase in β cell mass following initial β cell destruction. This results from both replication of surviving β cells and the budding of new islets from ducts. However, the regenarative process is not complete because these rats are glucose-intolerant in adult life. From these experiments with streptozotocin apparently a toxic insult to the β cells early in life can have dire consequences much later.

Streptozotocin apparently does trigger an autoimmune response against β cells in mice. Repeated subdiabetogenic doses of streptozotocin, administered to certain strains of mice can cause diabetes associated with islet inflammation and β cells destruction [179].

Our study corroborates the previous finding of blood sugar changes following streptozotocin injection. Following intraperitoneal injection of streptozotocin the blood sugar level started rising and the animal became diabetic and this increased blood sugar level was sustained till the death of the animal.

The idea of finding an element which may normalise the increased blood sugar level in streptozotocin induced experimental mice came from the works on Vanadium in lowering the increased blood sugar in diabetic mice.

Vanadium has been considered to be a potent environmental contaminant and extensive work has already been carried out on the toxic properties ascribed to vanadium in animals [180] and in humans following occupational and experimenal exposures [181].

Excessive cellular concentrations of vanadium result in toxic interactions which topple the cellular balance and the suggested regulatory role of vanadium causing increased cell death [182, 183, 184]. Later researches has shown vanadium to be capable of a beneficial behaviour under a thousand fold lower dose in comparison to the toxic higher doses.

This experience with vanadium led us to investigate another trace element selenium on the effect on experimental diabetes.

The objective of our study was to investigate the efficacy of low doses of selenium on the periods of progression of streptozotocin induced experimental diabetes in mice and which may be determined by some important markers that are responsible for biotransformation and detoxification.

A dose dependent study of different markers were done after feeding 3 different doses of selenium. It was found that Dose 2 i.e., 0.05 micro gm/0.1 ml was the most efficient dose in correction of different parameters as well as the blood sugar [113, 114].

Hepatic microsomal glucose-6-phosphatase (EC : 3.1.3.9) is potentially the most important enzyme involved in the homeostatic regulation of blood glucose concentrations. Substantial kinetic and genetic evidence indicates that glucose-6-phosphate hydrolysis in the glucogenic tissues is catalysed by a multi component system. It has been proposed that the active site of glucose-6-phosphatase in intact microsomes (microsomal fraction) is situated at the luminal

surface of the membrane and that a specific translocase (T_1) mediates entry of glucose-6-phosphate.

The level of glucose-6-phosphatase was elevated by 21.3% after production of experimental diabetes in mice and on selenium treatment the level fell to or below normal. Glucose-6-phosphatase catalyses the final step of glucose production by liver and kidney.

Miethke et al showed 150% increase of glucogenic enzyme glucose-6-phosphatase in diabetic mice [67].

Liu et al studied the effect of acute streptozotocin induced diabetes on hepatic microsomal glucose-6-phosphatase activity and m RNA expression in young, juvenile and adult rats. They found that acute streptozotocin diabetes increase expression of glucose-6-phosphatase m RNA and this contributes to the increased glucose-6-phosphatase activity seen with diabetes mellitus [87].

Burcelin et al showed 156% increase in glucose-6-phosphatase activity in streptozotocin diabetic rats. They proposed that in recent onset severly insulinopenic rats, an excessive glucose production via gluconeogenesis prevailed, mainly accounting for the concomitant hyperglycaemia. This excess glucose output cannot be attributed to liver insulin resistance : the gluconeogenic pathway is physiologically less sensitive than glycogenolysis to the inhibition by insulin [90].

Reddy et al studied the activity of glucose-6-phosphatase in liver under the influence of sepia shell extract in both normal and streptozotocin induced diabetic mice. Sepia shell possesses hypoglycemic effect. Activity of glucose-6-phosphatase was inhibited in both the normal and diabetic mice. The sepia shell extract enhances glycogenesis and reduces the formation of glucose from metabolic intermediates like pyruvate and glucose-1-phosphate and by suppressing gluconeogenesis [93].

Clore et al also found significantly increased hepatic glucose-6-phosphatase activity determined from freshly isolated microsomes in the type 2 diabetic patients. They concluded that increased endogenous glucose production which is a consistent feature of type 2 diabetes is mediated in part by increased glucose-6-phosphatase flux in type 2 diabetes [105].

Ghosh et al studied the effect of oral selenium on streptozotocin induced diabetic mice and found increase in glucose-6-phosphatase activity [115].

Dehydrogenation of glucose-6-phosphate to 6-phosphogluconate occurs via the formation of 6-phosphogluconolactone catalyzed by glucose-6-phosphate dehydrogenase, an NADP-dependent enzyme.

The level of glucose-6-phosphate dehydrogenase (EC : 1.1.1.49) was diminished by 20.8% in diabetic mice and on selenium treatment the level increased.

Shibib et al showed that Coccinia indica and Momordica charantia extracts lowered blood glucose by depressing its synthesis by enhancing glucose oxidation by the shunt pathway through activation of its principal enzyme glucose-6-phosphate dehydrogenase in streptozotocin induced hyperglycaemic rats [83].

Berg et al showed that administration of vanadate or Selenate in streptozotocin induced diabetic rats restore activity of glucose-6-phosphate dehydrogenase. Increase in cellular mRNA, responsible for increased synthesis of G6PD suggests the effect of insulin or mimetics (vanadate/ selenate in this case) occurs pretranslationally through gene regulation [116].

The level of pyruvic acid in liver was decreased to 78.9% in streptozotocin induced diabetic mice. On selenium treatment the level was almost normalised.

Reddy et al studied the status of pyruvate in liver under the influence of sepia shell extract in both normal and streptozotocin induced diabetic mice. The pyruvate concentration increased substantially in diabetic mice [93].

Glycogen is the major storage form of carbohydrate in animals and corresponds to starch in plants. It occurs mainly in liver (upto 6%) and muscle where it rarely exceeds 1%. However, because of its greater mass, muscle represents some three to four times as much glycogen store as liver.

The level of Glycogen in liver was decreased to 28.4% in experimentally induced diabetic mice. On selenium treatment the level of glycogen gradually recovered.

Miethke et al found that liver glycogen was reduced to 10% [67].

Khandelwal found marked decrease in liver glycogen and activities of glycogen-metabolizing enzymes in liver. Administration of oral sodium ortho vanadate restored the level [75].

Reddy et al studied the status of glycogen 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 [93].

Ghosh et al studied the effect of oral selenium on streptozotocin induced diabetic mice. Reversal of the decrease in hepatic glycogen was observed in diabetic mice on oral selenium[115].

Lactate formed by the oxidation of glucose in skeletal muscle and by erythrocytes, is transported to the liver and kidney where it reforms glucose, which again becomes available via the circulation for oxidation in the tissues. This process is known as the Cori cycle or lactic acid cycle.

Diabetes and selenium feeding had no effect on the lactic acid level in heart. A 40.3% diminution in the level of lactic acid in muscle was noted in diabetic mice. Selenium feeding increased the level. The level of lactic acid in liver fell by 35.8% in diabetic mice. The level rose to almost normal in selenium Dose 2 fed diabetic group. The level of lactic acid in blood rose to 55.5% in diabetic mice and the level fell with selenium treatment and came to almost normal value.

Higher lactic acid concentration in blood promoted the gluconeogenesis and the decrease in tissue level revealed maximum utilisation of glucose that has been taken up by the muscle cells through the oxidative pathway.

Kondoh et al while investigating the methylglyoxal bypass in animals found diabetic and starved rats had significantly higher level 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 [81].

Tormo et al investigated the lactate production by the intestine of normal and diabetic rats and found the lactate produced was significantly higher in diabetic than in normal rats [92].

No simple or successful method for the separation of succinic dehydrogenase (EC : 1.3.99.1) from cellular inclusions has been devised to date; hence, any assay for this enzyme has to be carried out in the presence of some or all components of the succinic dehydrogenase-cytochrome system. Cytochrome oxidase may be inactivated by the use of cyanide or by carrying out the estimation anaerobically. Under either of these conditions and with the addition of a hydrogen acceptor, the activity of the succinic dehydrogenase system may be estimated readily. The expression succinic dehydrogenase system refers to the system which catalyses the anaerobic oxidation of succinate and which probably includes cytochrome b in addition to succinic dehydrogenase and the hydrogen acceptor [185].

The level of succinic dehydrogenase in liver was increased by 25.9% in diabetic mice. Following selenium treatment the level fell and with Dose 2 fed mice the level came almost to normal.

Armstrong et al 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 [60].

Lenzen and Panten 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. They also found that 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].

These findings are contrary to our finding which may be due to change of experimental animal.

The distribution of LDH-isoenzymes (EC : 1.1.1.27) from homogenate supernatant of muscle solens detected five LDH-isoenzymes in normal rats. Adipose lactate dehydrogenase isozyme distribution was altered in streptozotocin diabetic and fasting rats resulting from a relative reduction of subunit A.

The changes of lactate dehydrogenase in serum on selenium treatment showed that the value in diabetic group becomes almost double the normal value.

Wohlrab and Schmidt found that 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].

Chang and Rothrock suggested that the redistribution of LDH-isozyme under these conditions is to prevent excessive accumulation of lactate in the tissue [61].

Increased LDH level may be due to cellular destruction that occurs in the diabetic pathology and posses an interference with the normal cardiovascular functions and is an associated risk.

Diabetes is more common now than it was before. Epidemiological studies have revealed diabetes to be a powerful factor for cardiovascular disease in general, particularly coronary disease. The most common lethal sequelae of diabetes was atherosclerotic large vessel disease. Diabetes predisposed subjects to all of the major atherosclerotic disease and coronary heart disease was the most common and most lethal. Incidence rate for cardiovascular disease is associated with increasing various risk factors including obesity, hypertension, cigeratte smoking etc. Although the incidence of diabetes on cardiovascular disease is greatly dependent on co-existent risk factors, there could be an independent effects of the diabetes in promoting the cardiovascular and coronary heart disease. This unique effect may derive from the disturbances in the metabolic effects of lipids and of the activities of enzymes like the lactate dehydrogenase and changes in the intrinsic catalytic behaviour of some of the enzymes that may be involved in the cardiovascular events. Fibrinogen status in diabetes may further contribute to this aspect. The study of the diabetic mice was undertaken to examine the level of fibrinogen, lactic acid, cholesterol, triglyceride, LDL, VLDL, HDL values and to measure the activity of lactate dehydrogenase and HMG CoA reductase (EC : 1.1.1.34) under the experimental diabetes condition; in order to assess the influence of diabetes on the cardiovascular system and to test the epidemiological observation in this regard.

The blood cholesterol level rose to 18.9% in diabetic mice in comparison to normal mice. With selenium treatment the level fell. In Dose 2 fed group the level came to normal level.

The level of serum LDL rose more than twice the normal value in diabetic mice. In Dose 2 fed animals the LDL level reached almost normal level.

The serum VLDL level rose 44.4% than the normal value, with production of diabetes. In selenium Dose 2 fed animals the level was almost normal.

The level of serum HDL was halved by induction of diabetes and in selenium Dose 2 fed animals the level approached near normal value.

The level of serum triglyceride was increased by 7 times in diabetic mice. Selenium feeding lowered the level.

The level of liver triglyceride was diminished by 7 times on induction of diabetes. In Dose 2 fed animals the level of liver triglyceride reached almost normal level.

In vanadium, selenium and vanadium & selenium fed normal mice the serum triglyceride level fell to half the normal level. But in diabetic groups vanadium alone failed to reduce the

triglyceride level. Selenium alone with Dose 2 is most effective in reducing the level. Also in the vanadium and selenium fed group the level is not satisfactorily reduced. So selenium Dose 2 is more effective than vanadium in respect to lowering of serum triglyceride level.

The activity of HMG-CoA reductase activity in liver was reduced by 22.8% in diabetic mice. In Dose 2 fed animals the activity neared the normal level.

Diabetes is associated with higher levels of triglyceride, cholesterol, lower HDL, increase in LDL and VLDL levels. These risk factors operate to enhance excess risk in diabetes. Our studies have confirmed the epidemiological observations (Framingham study) of William Kannel and others in respect of the variables involved in the cardiovascular derangements [186].

Diabetes is associated with lipolysis. Enhanced gluconeogenesis raise the serum triglyceride and mobilise fat from all tissues including liver which account for lowering of the liver triglyceride level in diabetic mice.

The unique effect of diabetes that is independent of the standard risk factors appears to be mostly explained by fibrinogen, mobilisation of lipid to blood and increase activity of LDH. Since the high risk of coronary heart disease in diabetes is concentrated in these with one or more associated cardiovascular risk factors, optimal management of cardiovascular sequelae require multivarious approach. Rational preventive measure must include raising HDL, lowering LDL, VLDL, decrease LDH activity instead of sole reliance on the correction of hyperglycaemia.

Verschoor et al have previously suggested that mechanisms other than reduced lipoprotein lipase activity might contribute to the defect in plasma removal of VLDL-triglyceride observed in insulin-deficient rats [65].

Goley et al found that plasma LDL-cholesterol was lower in alloxan induced diabetic rabbits [69].

Poucheret et al demonstrated that vanadyl sulfate can lower elevated blood glucose, cholesterol and triglycerides in a variety of diabetic models including the streptozotocin diabetic rat [100].

Sambandam et al found that diabetic rats had elevated triglyceride levels compared with control [106].

Douillet found that selenium or selenium or 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].

Xenobiotics are foreign toxic organic compounds, which must be detoxified by reactions making them less reactive or more water soluble and thus more amenable to excretion. Xenobiotic biotransformation end products are combined with endogenous molecules to form conjugates [187].

Glutathione (GSH) functions in the synthesis of important macromolecules and in the protection against reactive O_2 compound [188].

GSH was found to be effective in the management of various diseases of diverse aetiology. The level of glutathione in liver was raised by 39.1% in diabetic mice. With selenium Dose 2 feeding the level became almost normal.

The level of glutathione in blood in diabetic mice fell to almost half the normal level. With selenium Dose 2 feeding it was almost normal.

GSH is essential for the function of most if not all animal cells and maintains the functional and structural integrity of cells against the deleterious actions of metabolites and is maintained at a steady value to an autoregulatory mechanism [189].

The decrease in GSH level diorganises and disoriantes the mitochondria of the cell resulting in the reduced supply of ATP thereby limiting the optimum cellular and physiological functions in the diabetic plate. [190, 191, 192, 193].

GSH in blood reflects the GSH status in hepatocytes without considerable delay. But as GSH level is known to maintain the structural integrity of mitochondria, the decreased level of GSH may indicate the disorganisation of structure of mitochondria leading to the depletion of ATP synthesis. This finding is consistent with our previous result of inhibition in the activity of G-6-PDH limiting the supply of NADPH for maintaining the GSH in reduced state. Under such condition the cell might suffer from the lack of energy required for normal cellular and physiological processes. GSH is also an important cellular defense against oxidant injury and the significant role of GSH in the prevention of cellular lipid peroxidation has been well documented. The low level of GSH promotes an increase in lipid peroxidation resulting in a form of failure of adaptation on the part of GSH dependent defence mechanism against lipid peroxidation in rat liver microsomes to counteract the oxidative stress.

Mukherjee et al found a decline in GSH levels both in blood and liver compared with normal counterparts [84].

Kinalski et al found markedly diminished GSH level in the diabetic adult rats and their offspring in comparison to the control group [103].

Mukherjee et al showed marked decrease in glutathione. After 5 weeks of streptozotocin treatment, GSH level were reverted to normal by sodium selenite supplement [124].

Naziroglu et al indicated that intraperitoneally administered vitamin E and Se have significant protective effects on the blood, liver and muscle against oxidative damage of diabetes [130].

It is established that enhanced lipid peroxidation is followed by increased GSH oxidation to form oxidized glutathione (GSSG). In general, intracellular reduction of GSSG to GSH is mediated by glutathione reductase (EC : 1.6.4.2).

The level of glutathione reductase in liver fell by 20.9% in diabetic mice. With selenium feeding the level approached near normal level.

Godin et al showed in alloxan induced diabetic rats an increase in glutathione reductase activity [71].

Mukherjee et al showed a 20% decrease in GSH reductase activity in experimental diabetic condition [84].

Matkovics et al tested and compared antioxidant enzymes and glutathione reductase activity in streptozotocin diabetic animals. They concluded that streptozotocin treatment generally induces an oxidative predominance in tissues [97].

Glutathione-S-transferase (EC : 2.5.1.18) isoenzymes have a number of roles in the regulation of cellular metabolism. These include catalysis of the reduction of organic nitrates and hydroperoxides, steroid isomerization and the binding of non-substrate hydrophobic ligands such as steroid hormones, heme, bilirubin and numerous drugs. Multiple sub-units of GSH-S-T have been identified in a number of human and rat tissues and these can combine to form homo or heterodimeric isoenzymes. Upto 13 distinct isoenzyme subunits have been detected in human liver and lesser numbers in such tissues as kidney, testis, heart, lung, adrenals, brain, duodenum, ovary, placenta, spleen, eye lens and erythrocytes. This diversity and heterogenecity contributes to the inter tissue variation in several metabolic processes as well as the detoxification

of hydrophobic or electrophilic xenobiotics through catalytic conjugation with reduced glutathione (GSH) [194].

The GSH-S-T catalyze the conjugation of GSH with a variety of molecules that each have an electrophilic centre. These isoenzymes also exhibit selenium independent glutathione peroxidase activity and play an important role in the hepatic biotransformation and detoxification of xenobiotics. Consequently these enzymes may influence chemical carcinogenesis, mutagenesis and possible teratogenesis [195].

The level of glutathione-s-transferase in liver rose with production of diabetes and with selenium treatment the level continued to rise.

The glutathione-s-transferase is a multifunctional enzyme and it plays an important role in the detoxification of xenobiotic compounds with the help of GSH. The level of glutathione-s-transferase is found to be increased as compared to the control animals. The increase in this enzyme with the concomitant decrease in GSH level indicates accelerated detoxification pointing towards the possible accumulation of active metabolites and/or electrophilic molecules. Thus an increased utilization of GSH in the removal of toxic radical species through glutathione-s-transferase activity may eventually deplete the intracellular GSH pool.

Agius and Gidari demonstrated that streptozotocin increased the activity of mouse hepatic glutathione-s-transferases, but they suggested that this increase may be due to the direct action of streptozotocin and not as a result of the diabetic state the drug induces [66].

Thomas et al found reduced level of GSH-s-transferase in streptozotocin diabetic mice [72].

Mukherjee et al found an increase of GSH-s-transferase activity in experimental diabetic condition [84].

Raza et al found cytosolic glutathione concentration was decreased in diabetic rats and an increase of 20-30% in GSH-s-transferase activity in both diabetic and karela juice fed rats [94].

Giron et al fed diets with 5% olive, sunflower or fish oil for five weeks in streptozotocin diabetic rats and found in intestine GSH-S-T increased by diabetes [102].

Mukherjee et al found increase in glutathione-s-transferase activity after 5 weeks of streptozotocin treatment and reversion to normal by sodium selenite supplement [124].

Cytochrome P 450, Uridine diphospho glucoronyl transferase (UDPGT) and GSH-S-T enzyme systems are associated with the biotransformation for xenobiotics seen to be present in all vertebrates and has been best documented in mammals [196].

GSH-S-T catalyze the reaction between glutathione and a large variety of compounds bearing an electrophilic site [197].

UDPGT (EC : 2.4.1.17) on the other hand catalyzes the transfer of glucoronic acid to UDPGA to various phenolic, carboxylic acid and amine receptors.

The level of hepatic UDP-glucoronyl transferase rose on induction of diabetes and on selenium treatment went on rising.

Rouer et al measured the activities of UDP glucoronosyl transferase and cytosolic glutathione-s-transferase activities in the liver of streptozotocin induced diabetic mice. 2-3 fold increase was observed [63].

Numerous alterations in hepatic ultrastructure and metabolism occur during diabetes. These changes also seem to include the drug metabolizing enzymes as has been demonstrated for GSH-S-T and UDPGT and Cyt P 450 content [198].

Patterns in our observation show steady and progressive elevation of GSHT and UDPGT. Results indicate a simultaneous rythmicity of Cyt P 450 with UDPGT level followed by a decreased ATP synthesis. A failure of detoxification mechanism in the host under experimental diabetic conditions was evident that could induce a potentially increased risk of reactive metabolites under the condition of impaired detoxification capacities.

This study demonstrates the significantly altered levels of these drug metabolizing enzymes under the influence of pathophysiological condition like diabetes. Diabetes may act as proliferators of this altered state of xenobiotic degrading enzymes and could be expressive of intrinsic inductive potency.

The different rates of inductions of various drug metabolizing enzymes observed in the present study emphasise the need to carefully characterise the diabetic state for treatment with different drugs in different pathological conditions other than diabetes. Thus, considering the biological role of these different forms of enzyme, enough care has to be taken in balancing this status of enzyme activities, would be of interest in future.

Lipid peroxidation is primarily an outcome of the formation of free radicals, peroxides and superoxide anions. Active oxygen species interacts with unsaturated fatty acids present in phospholipid to initiate lipid peroxidation which is the major factor influencing the breakdown and turnover of biomembranes.

The level of lipid peroxidation in livers rose by six times in diabetic mice. With selenium Dose 2 feeding it approached the normal level.

The level of lipid peroxidation in brain rose by four fold in diabetic mice. With Dose 2 treatment the level fell almost to normal value.

The level of lipid peroxidation in kidney rose by five and half times in diabetic mice. With Dose 2 feeding it was almost normal.

The level of liver lipid peroxidation fell by one sixth part the normal value in both the vanadium and selenium fed normal mice groups. In both the vanadium and selenium fed diabetic groups the level fell by another one sixth part. Contrary to this result the level in vanadium plus selenium fed normal mice group only fell by a negligible amount and the level of liver lipid peroxidation came to normal in diabetic mice groups.

The present study documents a significant occurrence of an elevated lipid peroxidation in the tissues like brain, kidney and liver. The extent of increase in lipid peroxidation appear to be different in different tissues studied. This indicated that hyperglycaemia may affect different tissues in different fashions altering membrane lipid asymmetry that may have a role in the apparent reduced life span known to occur in diabetic patients.

Thomas et al depicted 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].

Jain et al showed that lipid peroxidation levels were significantly higher in RBC of diabetic rats than in controls [78].

Mukherjee et al found that a single intraperitoneal injection of streptozotocin caused a four, eight and seven fold increase in lipid peroxidation in brain, lever and kidney respectively [84].

Kinalski et al evaluated lipid peroxidation and scavenging enzyme activity in streptozotocin-induced diabetes and suggested 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].

Mukherjee et al showed increase in malondialdehyde levels in liver and blood after 5 weeks of streptozotocin treatment were reverted to normal by sodium selenite supplement [124].

Naziroglu et al determined the protective effects of intraperitoneally administered vitamin E and selenium on the lipid peroxidation as thiobarbituric acid reactive subsances (TBARS). They showed significant protective effects on the blood, liver and muscle against oxidative damage of diabetes [130].

Sato et al were the first to report increase level of lipid peroxide in plasma of diabetic patients [199]. Subsequent studies have confirmed this observation in diabetic patients and in animal and have suggested that hyperglycaemia may cause peroxidative injury to membrane.

Further, glutathione peroxidase has been demonstrated to participate in the conversion of toxic free radicals and organic hydroperoxides into hydroxy compounds in vivo and in vitro while oxidizing GSH into GSSG. In addition to glutathione peroxidase, catalase also acts as a scavenger of H_2O_2.

In our study diabetes and selenium feeding had no effect on catalase (EC : 1.11.1.6) level. This might ensure that the peroxidative damage due to H_2O_2 accumulation in the tissues is minimal in experimental diabetes [200].

Mukherjee et al found a marginal increase of catalase activity in streptozotocin induced diabetic rats [84].

Tatsuki et al studied the relationship between changes in lipid peroxides and those in catalase activity in pancreas, liver and heart of streptozotocin induced diabetic rats and suggested that the defense system in the pancreas to oxidative stress may be evoked in a early stage of streptozotocin induced diabetes [96].

Kakkar et al studied to identify whether oxidative stress occurs in the liver and pancreas in the initial stages of development of diabetes. They found increased catalase activity in liver and pancreas [98].

Dohi et al found selenium feeding in diabetic mice on liver and kidney catalase activity had no significant alteration [110].

The hepatic Cytochrome P 450 dependent drug metabolizing system catalyzes the metabolism of a wide variety of compounds such as xenobiotics, steroids, fatty acids and prostaglandins. Several forms of this family of enzymes exist in the untreated rat and several have been isolated and purified from chemically induced animals as well. Recently attention has been drawn to the constitutive forms of P 450 enzymes present in the untreated animal and presently 7 forms have been shown to exist in liver microsomes of the untreated male rat i.e. RLM 3 and RLM 5, UT-F, P 450f, RLM 2, RLM 5a and UT-H. Each of these forms is a separate and distinct Cytochrome P 450 based on such properties as absolute spectrum, molecular weight, isoelectric point, catalytic specificity and partial amino terminal amino acid sequence.

Although the levels of several of these constitutive forms have been shown to be unaltered, increased or decreased during treatment with chemical inducers or Cytochrome P 450, little is known regarding the regulation of these forms in altered physiological states not dependent on exogenous compounds. It is known, however, that certain hepatic microsomal P 450 dependent activities are altered as a result of streptozotocin induced diabetes and hypertension.

Studies have revealed that insulin dependent diabetes profoundly modulates the levels of inducible forms of Cyt P 450. The streptozotocin induced changes in P 450 related activity and apoproteins levels were successfully anatogonised by daily insulin therapy, demonstrating that the observed changes can be ascribed to the diabetic state rather than to the streptozotocin treatment [201].

The level of Cytochrome P 450 in liver fell by half in the diabetic group. With selenium feeding the level was more decreased.

Favreau and Schenkman measured the Cytchrome P 450 dependent hydroxylation of testosterone in hepatic microsomes of control, diabetic and insulin treated diabetic rats. 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].

Previous authors also made antibodies to monitor alterations in the content of the enzymes in livers of diabetic male rats. The results suggest there are atleast three types of responses by constitutents 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 in supplied and a rapid decline when insulin level declines and a restoration by insulin treatment [70].

Barnet et al concluded that insulin dependent diabetes induces proteins of the P 450 III and P 450 IV families [74].

Donahue and Morgan showed vanadate is capable of separating the effects of diabetes on expression of individual P 450 isozymes [76].

It is now becoming increasingly evident that the induction and regulation of the Cytochrome P 450 dependent mixed function oxidase is not determined solely by exposure to xenobiotics, but may also be trigerred by pathophysiological conditions, presumably resulting from an increase in the levels of an endogenous chemical with inducing properties.

Two major inducible families of Cytochrome P 450 are P 450 III and P 450 IV. The former is inducible by glucocorticoids, macrolide antibiotics and some imidazole containing antifungal agents. The P 450 IV family is inducible by xenobiotics such as the drug clofibrate and its analogues and the phthalate ester plasticisers.

In diabetes the chances of atherosclerotic disease are high and coronary heart disease was most common and most lethal. Recent studies have implicated fibrinogen in the occurance of cardiovascular disease (Framingham Study).

Level of fibrinogen in blood rose to 3 times in diabetic group. With selenium treatment the level fell and in Dose 2 the level of fibrinogen fell most. Increase fibrinogen values may influence thrombogenic tendency affecting a series of vascular events including the rheology of blood flow viscosity of the blood and distortability of red cells squeezing through the capillary circulation, seemed to be a reasonable condition. In addition, fibrinogen appears to influence the aggregation of platelets [202].

Diabetes mellitus in experimental animals was reported to be accompained by different changes in brain neurochemistry. Concerning monoamines, a decrease in a turnover rate of brain serotonin, dopamine and norepinephrine was found.

The level of monoamine oxidase (EC : 1.4.3.4) in serum rose by 43.9% in diabetic mice. In Dose 2 group the level came almost to normal.

Lackovic and Salkovic found the concentration of serotonin, dopamine and norepinephrine to be increased in the brain of a diabetic rat. But after an intracerebro ventricular administration of non diabetogenic doses of streptozotocin or alloxan brain monoamine change were similar to those observed in experimentally induced diabetic animals [79].

Acetyl cholinesterase (EC : 3.1.1.7) catalyzes the hydrolysis of acetylcholine and other acetic acid esters, as well as certain esters and acyl halides of substituted phosphoric, carbamic and sulfonic acids. In view of the diversity of these molecules, it is generally believed that their binding patterns differ, while the hydrolytic part of the enzymic reaction remains the same. The widely used (pesticides) esters of phosphoric acid and carbamic acid are generally believed to exert their anticholinergic toxic effects essentially in a similar manner.

Although acetyl cholinesterase shares its nucleophilic activity of a serine hydroxyl group with many other hydrolases, notably chymotrypsin, its substrate specificity stems from the still ill defined binding phenomenon. The large variation in the chemical structure among the substrates and inhibitors that react with the enzyme precluded any attempt to oversimplify or unify the binding patterns [203].

The level of acetylcholinesterase in brain in diabetic group fell by 13 times. With selenium feeding the level rose.

Dash et al found hyperglycemia due to experimental diabetes induced in rats, causes a decrease in the activity of acetylcholinesterase in brain regions and heart [80].

Wahba et al estimated the activities of acetylcholinesterase in the seminal vesicle and in urinary bladder in streptozotocin induced diabetic rats. They found increase in the enzyme in detrusor muscles and insignificant result in seminal vesicles [82].

Urea biosynthesis is divided into four stages : (1) transamination, (2) oxidative deamination of glutamate, (3) ammonia transport, (4) reactions of the urea cycle.

The blood urea level rose by 20.4% in diabetic mice. With selenium treatment the levels rose and in Dose 2 fed group the level rose more than twice the normal.

With production of experimental diabetes the content of selenium in pancreas was increased by more than 10 times the normal value. The level of selenium in liver rose by one and half times in diabetic mice. In brain there is marginal lowering of selenium content and in blood also there is lowering of the selenium level.

Mice has 38 autosomes and 2 sex chromosomes. Mitotic chromosomes are observed at metaphase stage. Metaphase plates contained 40 chromosomal elements. An ideogram karyotype of mice somatic cells are made by arranging them according to their decreasing order in size. It has been shown that 40 chromosomes and the diploid chromosome set, though appeared to be

very similar to each other can be subdivided into 5 distinct groups, each including chromosomes of more or less similar sizes.

In our study no chromosomal abnormality was found on production of diabetes and on selenium treatment no change occured.

Homogenates of tissue from mature animals that do not show a rapid rate of mitosis readily undergo lipid peroxidation (e.g. brain, liver, kidney) whereas those tissues that are not susceptible to lipid peroxidation (e.g. testis and intestinal epithelium) undergo rapid cell division [204].

In histopathology of pancreas the islets of Langerhans showed a variety of changes, but in about one-third of pancreas no abnormality can be detected. In early diabetes a lymphocyte infiltrate (insulitis) may be present. In late stages the β cells show vacuolation due to an accumulations of glycogen and atrophy of the islets with replacement by amyloid or fibrous tissue.

Selenium treatment could not reverse the process of streptozotocin induced atrophy of the islets.

Low levels of dietary selenium do not cause cancer, but decreased levels of Se increase susceptibility to cancer given a carcinogenic exposure.

Slonim et al found that the mice fed on Vit E, selenium deficient diet, showed increased susceptibility to diabetes development by ordinarily non-diabetogenic doses of streptozotocin [109].

Douillet et al studied the effect of selenomethionine along with Vit E on platelet activity in diabetic rats in vitro and found a reduction in platelet thrombin, ADP induced aggregation, in adhesiveness to fibronectin and in sorbitol content [123].

Kowluru et al found dietary supplementation into antioxidants offers a means to inhibit multiple hyperglycaemia induced retinal metabolic abnormalities [125].

Naziroglu et al found Vit C to outweigh the effect of Vit E and selenium against oxidative damage to lens [126].

Kowluru et al suggested that antioxidants inhibit abnormal metabolic processes that may contribute to the development of cardiac disease in diabetes [127].

Goemen et al stated that 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].

Kowluru et al stated that 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].

Reddi et al found that selenium supplementation to diabetic rats prevents not only oxidative stress but renal structural injury as well [132].

Kowluru et al found that long term administration of antioxidants can inhibit the development of the early stages of diabetic retinopathy [134].

In our study on the role of selenium in experimental diabetes we found that many parameters are normalised by selenium feeding in streptozotocin induced diabetic mice. But some parameters are not corrected, even some got worse.

So we may conclude thus that selenium is not a true replacement of insulin. A dose responsive action occurs on diabetes. The best dose has to be determined and an adjuvant has to be found to provide together the best result on diabetes.

This idea is leading towards the role of mixture of antioxidants for protection against the complication of diabetes.

Parameters	Statistical analysis
1. Blood Sugar	Dose-2 - deviation from normal is insignificant.
2. Glucose 6 phophatase in liver	Dose-2 - produces most significant reduction.
3. Glucose 6 po_4 dehydrogenase	Dose-2 - deviation from normal is insignificant.
4. Pyruvic acid in liver	Dose-2 - deviation from normal is insignificant.
5. Glycogen in liver	Dose-2 - deviation from normal is insignificant.
6. Lactic acid in blood	Dose-2 - produces most significant reduction.
7. Lactic acid in heart	No change.
8. Lactic acid in muscle	Dose-2 - produces insignificant deviation but at 3%.
9. Lactic acid in liver	Dose-2 - produces insignificant deviation but at 2%.
10. Succinic dehydrogenase in liver	Dose-2 - produces insignificant deviation from normal.
11. Blood cholesterol	Dose-2 - produces insignificant deviation from normal.
12. Serum LDL	Dose-2 - produces insignificant deviation from normal.
13. Serum VLDL	Dose-2 - produces insignificant deviation from normal.
14. Serum HDL	Dose-2 - produces insignificant deviation from normal.
15. Serum triglyceride	No dose shows significant improvement.
16. Liver triglyceride	No dose shows significant improvement.
17. HMG-CoA reductase in liver	Dose-2 - produces insignificant deviation from normal.
18. Glutathione in liver	D-2 & 3 - produces insignificant deviation from normal.
19. Glutathione in blood	D-2 - produces most insignificant deviation from normal.
20. Glutathione reductase in liver	Dose-2 - produces insignificant deviation from normal.
21. Glutathione S-transferase in liver	No significant variation from normal except Dose-3.
22. Hepatic UDP-glucoronyl transferase	No doses produce insignificant deviation from normal.
23. Lipid peroxidation in liver	Dose-2 - produces insignificant deviation but at 3%.
24. Lipid peroxidation in brain	No dose produces significant improvement to normal.
25. Lipid peroxidation in kidney	No dose produces significant deviation towards normal.
26. Catalase in liver	No change.
27. Cyt P-450 in liver	No dose show significant improvement.
28. Fibrinogen in blood	D-2 - produces insignificant deviation to normal at 6%.
29. Monoamine oxidase in serum	Dose-2 - produces insignificant deviation from normal.
30. Acetyl choline esterase in brain	No significant improvement.
31. Urea in blood	No dose show significant improvement.