Summary
SUMMARY

Diabetes mellitus is characterized by derangements in carbohydrate, protein and lipid metabolism caused by the complete or relative insufficiency of insulin secretion and/ or insulin action. There are two main forms of diabetes; type 1 (Insulin dependent diabetes mellitus) and type 2 (Non-insulin dependent diabetes mellitus). Type 1 diabetes mellitus is due to autoimmune-mediated deterioration of pancreatic β-cells, resulting in absolute insulin deficiency. Type 2 diabetes mellitus is far more common and characterized by insulin resistance and/ or abnormal insulin secretion, either of which may predominate. People with type 2 diabetes mellitus are not dependent on exogenous insulin, but may require it for the control of blood glucose levels, if this is not achieved with diet alone or oral antidiabetic agents. Insulin resistance is a state of reduced insulin sensitivity, an inability of insulin to lower plasma glucose levels through suppression of hepatic glucose production in liver and stimulation of glucose uptake in skeletal muscles and adipose tissue. The coexistence of insulin resistance and hyperinsulinemia appears to contribute directly or indirectly to many other disorders, such as dyslipidemia, hypertension, atherosclerosis and a procoagulant state, linking together with insulin resistance, which has now assumed the status of syndrome, namely Syndrome X.

Alcohol abuse is thought to be a risk factor for the cause of liver damage, dyslipidemia and insulin resistance in human beings nevertheless, the mechanisms by which alcoholic beverages could mediate insulin resistance in target organs are not very well known due to lack of suitable experimental models. In the present study, alcoholism and its impact on carbohydrate metabolism has been studied in detail in albino rats. The Charles Foster strain of albino rats was randomly divided into groups. Rats of control group received sucrose (10 %) in addition to normal pellet diet; this was done primarily to maintain isocaloric balance. The experimental group of rats received 40 % ethanol at an oral dose of 3.76 g/kg body weight for 45 consecutive days.

Activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, acid phosphatase, γ-glutamyl transferase, sorbitol dehydrogenase and alcohol dehydrogenase were found significantly higher in the serum of alcohol-fed rats. The fasting blood glucose and serum insulin levels were also found significantly higher in alcohol fed rats. The serum levels of C-peptide and glucagon like peptide-1 (GLP-1) were also significantly increased after six weeks of alcohol feeding. The level of leptin in
alcohol fed rats was also found to be increased whereas the level of adiponectin in alcohol fed rats was observed significantly lowered.

The serum levels of total cholesterol and triglyceride were found significantly higher in alcohol-fed rats. The levels of HDL-cholesterol and LDL-cholesterol were also observed significantly higher in alcohol-fed rats. Apolipoprotein profiles (Apo A-II, Apo C-II and Apo E) were also observed significantly higher in the serum of alcohol-fed rats. Rats of alcohol fed group also had significantly higher level of ethanol in their blood.

Glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (PEPCK) are the major regulatory enzymes of gluconeogenesis pathway. Their activities get enhanced in the liver of alcohol fed rats compared to sucrose fed rats. Glucose-6-phosphatase activity was also found to be increased in the kidney and muscle of alcohol fed rats. PEPCK activity was observed to be increased in the kidney whereas found to be decreased in the muscles of alcohol fed rats. Glycogen phosphorylase activity was found to be increased significantly in the liver of alcohol fed rats. The glycogen phosphorylase activity was also found higher in the kidney and muscles of alcohol fed rats compared to the counterparts from sucrose fed rats. Phosphofructokinase is one of the regulatory enzymes of glycolysis; its activity was found to be diminished in alcohol fed rats. Pyruvate kinase plays an important role in the utilization of glucose and thereby helping the glucose uptake. Its activity was observed to be decreased in the liver, kidney and muscle of alcohol fed rats. The activity of lactate dehydrogenase was also found to be decreased in the tissues of alcohol fed rats compared to normal control group. Protein tyrosine phosphatase activity was found to be modulated in alcohol fed rats especially in the insulin sensitive tissues like liver and muscle. Its activity was found to be increased in the liver and muscles but no significant difference was observed in kidney following ethanol feeding to albino rats. This alteration in enzyme activities may be due to defect in carbohydrate metabolism of the rats induced by chronic ethanol feeding possibly it is doing so by increasing the activities of glucose-6-phosphatase and PEPCK, one of the main reasons of achieving hyperglycemia in alcoholic rats. The alcohol dehydrogenase activity was found to be decreased significantly in the liver of alcohol fed rats compared to normal control group whereas its activity was found to be slightly higher in the kidney and muscle of alcohol fed rats compared to control i.e. sucrose fed rats.

Significant increase in the serum insulin level and impaired glucose tolerance in alcohol-fed rats might be due to insulin resistance caused by ethanol. After three weeks
of ethanol feeding to Charles Foster rats, significant amount of deterioration occur in the oral glucose tolerance curve as compared to control group fed with standard pellet diet and sucrose to balance isocaloric intake. This development in the glucose intolerance becomes progressive every week deviating from the normal pattern of glucose tolerance. There is due to an increase in the fasting blood glucose levels as compared to normal rats, which reaches up to 8-10 mM 30 min post glucose load of 2.5 g/kg body weight. The present study thus confirms that chronic ethanol feeding to rats also impairs their glucose tolerance and carbohydrate metabolism like that of human beings.

Numerous molecules have been reported to possess antidiabetic activity and mechanisms for the antidiabetic action of such molecules have been described. Because of many side effects and doubt about the efficacy and safety of the present day available oral hypoglycemic agents promoted a search for safer and more effective drugs for the treatment of diabetes in particular type 2 diabetes mellitus. A large number of synthetic molecules belonging to several chemical series were evaluated for their antihyperglycemic activity in different animal models developed for diabetes mellitus and insulin resistance. In the present study antihyperglycemic effect of synthetic compounds of nearly ten chemical series was studied in sucrose loaded and sucrose challenged streptozotocin-induced diabetic rats. Sucrose loaded rat model was primarily employed to identify the lead antihyperglycemic molecule. Streptozotocin-induced diabetic rat model was later used to confirm the antihyperglycemic activity of these lead molecules under diabetic conditions.

A total of two hundred sixty synthetic compounds belonging to nearly ten different chemical class/series and their derivatives were tested for their antihyperglycemic property on postprandial hyperglycemia in normal rats; out of which a total of seventy compounds showed significant (p<0.05) antihyperglycemic effect in sucrose loaded rats and compared with four known standard antidiabetic drugs i.e. glibenclamide, gliclazide, metformin and acarbose.

The derivatives of carbamate and chalcone were found very effective in lowering blood glucose level as evidenced from the fact that it inhibits the postprandial rise in hyperglycemia in sucrose loaded rats. These lead molecules also prevent the rise in blood glucose level even in streptozotocin-induced diabetic rats post sucrose challenge. Some of the derivatives belonging to other chemical series i.e. phenylethylurea, tetrazole,
Summary

flavonoid, naphthooxazole, xanthone, pregnene and propiophenone also showed significant (p<0.05) antihyperglycemic effect in sucrose loaded rat model.

The synthetic compounds showing significant antihyperglycemic activity were further tested in sucrose challenged streptozotocin-induced diabetic rat model for the confirmation of antihyperglycemic potential under diabetic conditions. A total of thirty synthetic compounds belonging to various chemical series were tested in this animal model for diabetes mellitus; out of which fifteen synthetic compounds namely S-001-115, S-002-853, S-002-857, S-003-1128, S-003-353 D2, S-003-354 D1, S-003-849, S-003-1020, S-001-284, S-001-285, S-002-106, S-003-446, S-003-447, S-001-8 and S-003-309 showed significant (p<0.05) antihyperglycemic activity in this animal model. Lowering of blood glucose levels in the streptozotocin-induced diabetic was evident from 1 h which continued till 5 h. The peak lowering in most of the cases was observed during 3 to 5 h post treatment.

The db/db mouse is a well-characterised model of type 2 diabetes mellitus. The major deficiency of the C57BL/KsBom-db mouse (db/db) is lack of a functional leptin receptor. This leads to defective leptin signalling and a complete lack of feedback from leptin. Both hypothalamic NPY content and secretion are consequently elevated, and this result in hyperphagia and decreased energy expenditure, obesity, insulin-resistance, hyperinsulinemia, hyperglycemia and dyslipidemia. A total of twelve synthetic compounds which were found active in both the above said animal models for diabetes mellitus were also evaluated to check their antidiabetic and insulin resistance reversal potentials in a genetic diabetic animal model i.e. db/db mice. Out of twelve synthetic compounds evaluated seven molecules (S-002-853, S-002-857, S-003-1125, S-003-1130, S-001-469, S-001-471 and S-003-447) belonging to three different chemical series were found active in db/db mice. These molecules showed significant antihyperglycemic activity on day 6 and on oral glucose tolerance test i.e. on day 10. Metformin, a known antidiabetic drug available in the market was also tested in this animal model.

Identification of new in vitro target based antidiabetics is the basic need for today's therapeutics for diabetes mellitus. Glucose-6-phosphatase, glycogen phosphorylase and protein tyrosine phosphatase are among the important in vitro targets for diabetes mellitus and insulin resistance. Search for the inhibitors of these enzymes were one of the major objectives of our studies. Glucose-6-phosphatase, an enzyme that operates the penultimate step of gluconeogenesis has been identified as one of the
important targets for diabetes mellitus in recent years. Starvation and diabetes mellitus causes 2-3 fold increases in glucose-6-phosphatase activity in the liver. The potential inhibitors of glucose-6-phosphatase can be developed as antidiabetic agents. A total of 170 crude extracts of plants and 60 synthetic compounds (bicyclic biaryls and ureidoalkanoic acid derivatives), were tested for their inhibitory action on glucose-6-phosphatase at 100 μM concentration. The seven plant extracts (4469, 4471, 4504, 4512, 4520, 4527 and 4569) and sixteen synthetic compounds (S-002-260, S-002-262, S-002-264, S-003-178, S-003-180, S-003-265, S-003-266, S-003-268, S-003-273, S-003-276, HTS-13, HTS-14, HTS-16, HTS-17, HTS-20 and HTS-29) showed more than 50 % inhibition on glucose-6-phosphatase from rat liver at 100 μM concentration. The per cent inhibition of glucose-6-phosphatase was also compared with the standard i.e. sodium orthovanadate which could inhibit glucose-6-phosphatase to the tune of 18.6 % at 100 μM concentration.

Another in vitro target for controlling diabetes mellitus is glycogen phosphorylase, an enzyme that catalyses the release of glucose from glycogen. Glycogen phosphorylase inhibitors could function as new hypoglycemic agents for the treatment of type 2 diabetes mellitus. Inhibitory effect of crude extracts of plants and synthetic compounds (bicyclic biaryls and ureidoalkanoic acid derivatives) on glycogen phosphorylase was tested at 100 μM concentration. A total of 170 crude extracts of plants and 40 synthetic compounds were tested for their inhibitory effect on glycogen phosphorylase. Nine plant extracts (4146, 4301, 4324, 4357, 4412, 4434, 4452, 4500 and 4504) and twelve synthetic compounds (S-002-257, S-002-258, S-002-260, S-002-262, S-002-263, S-003-265, S-003-268, S-003-269, S-003-273, S-003-277, S-003-280 and S-003-281) showed more than 50 % inhibition on glycogen phosphorylase from rat liver at 100 μM concentration.

Another important in vitro target for diabetes mellitus and insulin resistance is protein tyrosine phosphatase (PTP). Tyrosine phosphorylation of cellular proteins by protein kinases seems to play a profound but complicated role in β-cell growth, development and secretion. Protein tyrosine phosphorylation is controlled not only by tyrosine kinases but also by the activity of protein tyrosine phosphatases that dephosphorylate phosphotyrosine residues, an important step in signal transduction pathway in the insulin secretion/ action, which gets disturbed in diabetes mellitus and insulin resistance. An inhibitor of protein tyrosine phosphatase can be used effectively to
reverse insulin resistance and consequently increase the insulin sensitivity for tissues like liver and muscle. Protein tyrosine phosphatase-1B, an enzyme of high importance, plays an important role in carrying out insulin signaling cascade together with protein tyrosine kinase. In normal circumstances there is a synchronization between these two enzymes i.e. protein tyrosine kinase and protein tyrosine phosphatase. In the case of insulin resistance the activity of protein tyrosine phosphatase-1B was found to be increased because of the high expression of PTP gene. Therefore, for the treatment of insulin resistance and non-insulin dependent diabetes mellitus one has to inhibit the activity of protein tyrosine phosphatase-1B. An inhibitor of protein tyrosine phosphatase can be used effectively to reverse insulin resistance and consequently increased insulin sensitivity for liver and muscles.

Inhibitory effect of few compounds of combinatorial libraries and derivatives of furanoflavonoid were checked on protein tyrosine phosphatase. Six library compounds i.e. L-000-17-8, L-000-17-19, L-000-17-25, L-000-17-31, L-000-17-40 and L-000-17-46 showed more than 50% inhibition on this enzyme at 100 μM concentration. Six synthetic compounds namely S-003-691, S-004-280, S-004-281, S-004-283, HTS-592 and HTS-727 showed more than 50% inhibition at 100 μM concentration. Their per cent inhibition were also compared with the known mammalian inhibitors i.e. peroxovanadate that could maximally inhibit protein tyrosine phosphatase to around 66.7% at 100 μM concentration.

The present study showed the elevation in blood glucose and serum insulin profiles in Charles Foster albino rats following consecutive feeding of ethanol. Glucose tolerance was found largely impaired in these rats when fed with ethanol for four consecutive weeks. An increase in the levels of serum cholesterol and triglycerides were also observed in alcohol fed rats compared to normal sucrose fed rats. Elevated levels of blood glucose and serum insulin of alcohol fed rats suggest the resistance of these animals towards insulin. This interesting animal model of insulin resistance was later used for the evaluation of antihyperglycemic compounds for their insulin resistant reversal property.

Two synthetic compounds (S-001-471 and S-002-857) and a standard drug metformin which were found active in all the above said animal models for diabetes mellitus were also tested in this newly developed insulin resistance model of alcohol fed rats. Alcohol fed rats treated with S-001-471 and S-002-853 showed significant
improvement on their oral glucose tolerance curve after two weeks treatment compared to control group. Lowering in blood glucose profile and significant reduction in serum insulin levels was observed in alcohol fed rats when treated with S-001-471 and S-002-853 for two weeks. A significant improvement on lipid profiles was also observed in alcohol fed albino rats treated with S-001-471 and S-002-853. Metformin lowers all the above said elevated parameters significantly in alcohol fed rats at the same dose level and also having significant improvement on glucose tolerance curve after two weeks treatment.

Both synthetic antihyperglycemic compounds S-001-471, S-002-853 and standard drug metformin showed promise in alcohol fed rat model as indicated by improvement on impaired fasting blood glucose profile, impaired glucose tolerance and normalization of the elevated serum insulin levels, as well as improvement on lipid profile responsible for insulin resistance and type 2 diabetes mellitus in this animal model.

Insulin resistance was measured using HOMA index (homeostasis model assessment). It is a mathematical model utilizing ratio of fasting glucose (mM)/fasting insulin (µU/ml) that has been used to indicate the degree to which they combine to give hyperglycemia with low, normal or raised basal plasma insulin concentrations. S-001-471 and S-002-853 were found to correct hyperinsulinemia by decreasing fasting serum insulin in alcohol fed rats prevented the rise in serum insulin levels following glucose load. Insulin resistance was found to be established as calculated by HOMA index formula using fasting glucose and fasting serum insulin. Alcohol fed rats exhibited insulin resistance index 4.56 which reduced significantly after the treatment with both S-001-471 and S-002-853. It is again proved that S-001-471 and S-002-853 possess the property of reversing insulin resistance and enhancing insulin action by increasing insulin sensitivity towards glucose. This is being the first report about the existence of antihyperglycemic and insulin resistant reversal property in derivatives belonging to propiophenone and flavone chemical series.