SECTION-D

SUMMARY & CONCLUSION
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Diabetes mellitus is a heterogeneous group of metabolic disorders characterized by high blood glucose level. The pancreatic β-cells and its secretary hormone i.e. insulin are central in the pathophysiology of diabetes. Type 1 or insulin dependent diabetes mellitus results from an absolute deficiency of insulin due to autoimmune destruction of the insulin producing pancreatic β-cells. In type 2 or non-insulin dependent diabetes mellitus, muscle and fat cells are 'resistant' to the action of the insulin and compensatory mechanisms that are activated in the β-cell to secrete more insulin are not sufficient to maintain blood glucose levels with in a normal physiological range. This state is also linked to other common health problems, such as obesity, polycystic ovarian disease, hyperlipidemia, hypertension and atherosclerosis. The epidemic of type 2 diabetes and impaired glucose tolerance are main causes of morbidity and mortality world wide. The pathophysiology of insulin resistance involves a complex network of signaling pathways activated by the insulin receptor, which regulate intermediary metabolism and its organization in cells. All forms of diabetes are characterized by chronic hyperglycemia and the development of diabetes specific micro-vascular pathology in the retina, renal glomerulus and peripheral nerves. As a consequence of its microvascular pathology, diabetes is a leading cause of blindness, end stage renal disease and a variety of debilitating neuropathies. Diabetes is also associated with accelerated atherosclerotic macrovascular disease; affecting arteries that supply blood to the heart, brain and lower extremities. As a result, patients with diabetes have a much higher risk of myocardial infarction, stroke and limb amputation. Hyperglycemia and insulin resistance both seem to have important roles in the pathogenesis of microvascular as well as macrovascular complications. Insulin resistance is a state of reduced insulin sensitivity, an inability of insulin to lower plasma glucose level through suppression of hepatic glucose production 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.

At present, therapy for type 2 diabetes relies mainly on several approaches intended to reduce the hyperglycemia itself; sulphonylureas, which increase insulin release from pancreatic islets; metformin, which acts to reduce hepatic glucose production;
PPAR-\(\gamma\) agonists (thiazolidinediones), which enhance insulin sensitivity; \(\alpha\)-glucosidase inhibitors, which interfere with gut absorption; and insulin itself, which suppresses glucose production and augments glucose utilization. These therapies have limited efficacy, limited tolerability and significant mechanism based side effects. Of particular concern is the tendency for most treatments to enhance weight gain. Several current approaches are also associated with episodes of hypoglycemia and few of the available therapies adequately address underlying defects such as obesity and/or insulin resistance. A problem particular to the sulphonylureas is that many patients who respond initially become refractory to treatment overtime (secondary failure). Thus newer approaches are desperately needed.

Since ancient times, plants have been an extemporary source of medicine. Ayurveda and Indian literature mention the use of plants in treatment of various human ailments. India has about 45 thousand plant species and among them several thousands have been claimed to possess medicinal properties. According to an estimate of World Health Organization (WHO) nearly 8% population of the developing countries relies on traditional medicine, mostly plant drugs, for their primary health care needs. In light of these evidences, an attempt was made to identify some new antidiabetic agents using experimentally validated animal models of diabetes.

In the present study, we attempted to develop \(db/+\) mice as an alternative model for antidiabetic drug discovery programme. Investigation of newer formulations with antidiabetic properties is the need of time and mouse has become a popular model for different antidiabetic research programme because it is genetically well defined, has short generation time and environmental factors can be controlled easily in the laboratory because of small size. In \(db/db\) mice, a single gene mutation in leptin receptor gene led to diabetic condition, and recently it has become an important animal model of type-2 diabetes. The mutation is a unit autosomal recessive with full penetrance, and causes metabolic disturbances only in homozygous condition. The heterozygous counterpart \((db/+\)) remains normal and does not become diabetic at any stage of its life span, rather remaining a carrier of the mutation. The only importance of these heterozygous mice is in breeding. In conclusion, streptozotocin-induced diabetic \(db/+\) mice showed most of the characteristics of diabetes mellitus and can be used as an alternate \textit{in-vivo} model for antidiabetic drug discovery research. However, further evaluation of the model in term of molecular mechanism and its resemblance with human diabetes needs further investigation.
The *in-vivo* antihyperglycemic activity of large number of phytoconstituents and plant based synthetic isoflavone derivatives were evaluated in different animal models developed for diabetes mellitus and insulin resistance. In the present study antihyperglycemic effect of these compounds was initially studied in STZ-induced β-cell damaged diabetic rats. This rat model was primarily employed to identify the lead antidiabetic molecules. The antihyperglycemic activity of phytoconstituents-withanolides (K037 & K041), α-amyrin acetate (α-AA) and 4-hydroxypipeolic acid (4-HPA) isolated from the fruits of *Withania coagulans*, aerial roots of *Ficus bengelensis* and seeds of *Peganum harmala* respectively were evaluated in STZ-induced β-cell damaged diabetic rats. Treatment of these compounds showed significant blood glucose lowering effect in diabetic rats which was comparable the blood glucose lowering effect of known standard antidiabetic drug metformin.

The synthetic derivatives of isoflavone RS-853 and DL-857 and their resolved enantiomer S-853/R-853 and D-857/L-857, were found very effective in lowering blood glucose level, they inhibit the postprandial rise in hyperglycemia in STZ-induced diabetic rats post sucrose challenge comparable to that of metformin. Following the confirmation of antihyperglycemic activity in STZ-induced diabetic rats, these antidiabetic lead molecules were further studied in the ideal model of type 2 diabetes (*db/db* mice).

The *db/db* mouse is a well-characterised model of type 2 diabetes mellitus. The major deficiency of the *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 neuropeptide Y content and secretion are consequently elevated and this results in hyperphagia, decreased energy expenditure, obesity, insulin resistance, hyperinsulinemia, hyperglycaemia and dyslipidemia in *db/db* mice. The *db/db* mice at 12 weeks of age exhibited most of the human characteristics of type 2 diabetes including hyperglycemia in the fasting and fed states, hyperinsulinemia and insulin resistance. The supplementation of phytoconstituents; K037 & K041, α-AA and 4-HPA, and synthetic isoflavone derivatives (RS-853 & DL-857) and their resolved enantiomer S-853/R-853 and D-857/L-857 significantly improved postprandial glucose control as assessed by an OGTT in the *db/db* mice. Furthermore, treatment of these antihyperglycemic agents significantly lowered the level of plasma insulin, while increasing the ability of insulin to lower glucose in OGTT, demonstrating improved insulin sensitivity. Interestingly, the supplementation of these antihyperglycemic agents in the *db/db* mice normalized the increased plasma triglycerides,
total cholesterol and LDL-cholesterol level and attenuated lipid accumulation in the plasma of db/db mice.

Biochemical studies showed that the level of key regulatory enzymes of carbohydrate metabolism altered in diabetic db/db mice. Low hepatic GK, PFK and PK activities is also reported to favor the release of glucose synthesized by gluconeogenesis into the circulation. Hepatic gluconeogenesis is also crucial to the maintenance of fasting hyperglycemia and was observed to be high in db/db mice. The supplementation of withanolide (K037 & K041), α-AA and 4-HPA as well as synthetic isoflavone derivatives (RS-853 & DL-857) and its resolved enantiomers (S-853/R-853 & D-857/L-857) significantly elevated the activity of hepatic GK, PFK and Pyruvate kinase in db/db mice. The G-6-Pase, fructose-1, 6-bisphosphatase and PEPCK are the key enzymes that control gluconeogenesis and glucose output from the liver, and their gene expressions were increased in db/db mice. In the study, phytoconstituents and synthetic isoflavone derivatives caused a marked reduction in the hepatic PEPCK, F-1, 6-BPase and G-6-Pase activity in db/db mice, indicating a decreased hepatic glucose production. Based on these results, these antihyperglycemic agents seemed to suppress the hepatic glucose output by enhancing hepatic glucose utilization and inhibiting glucose over-production in db/db mice.

Although the exact mechanism(s) by which these phytoconstituents withanolide (K037 & K041), 4-HPA and α-AA, plant based synthetic isoflavone derivatives; RS-853 and their enantiomer (S-853/R-853) and DL-857 and their enantiomers D-857/L-857) lowered the blood glucose level in the diabetic rats is still being worked out, however several possible hypothesis that may involved in the therapeutic action can be considered on the basis of present results. Firstly, these antihyperglycemic agents may exert their therapeutic effect through modulation of insulin secretion, perhaps by rejuvenating the insulin secreting β-cells in the islets of langerhans, thus increasing the capacity of insulin secretion. Secondly, these antihyperglycemic agents may have insulin-like effect wetherby they stimulate peripheral insulin sensitivity during diabetes and third, these antihyperglycemic agents stimulate or inhibit the regulatory enzymes of carbohydrate metabolism pathways. It is unclear at this juncture whether these antihyperglycemic agents follow such mechanisms of action in their therapeutic role. However, further detailed studies are necessary before firm conclusions can be drown on the mechanism(s) of action of these novel antihyperglycemic agents as to whether these antihyperglycemic agents follow similar signaling biochemical routes such as those taken by insulin during therapy.
In conclusion, our studies show that the phytoconstituents; withanolide (K037 & K041), α-AA and 4-HPA) and plant based synthetic isoflavones derivatives (RS-853 and S-853/R-853 enantiomer and DL-857 and D-857/L-857 enantiomer) exerted promising antihyperglycemic effects in STZ-induced β-cell damaged diabetic rats and db/db mice. Thus, these antihyperglycemic agents can be developed as antidiabetic lead molecules and further studies carried out in this direction to find out mechanism(s) of action of lead molecules may lead to newer molecules in treatment of diabetes.

Fig: Possible points of action for blood glucose lowering effect of phytoconstituents (K037, K041, 4-HPA and α-amyrin acetate) and isoflavone derivatives (RS/S-, R- and DL/D-, L-) in the pathway of carbohydrate metabolism. 1; Glycogen Phosphorylase, 2; Glucose-6-phosphatase, 3; Glucokinase, 4; Fructose-1, 6-bisphosphatase, 5; Phosphofructokinase, 6; Phosphoenolpyruvate Carboxykinase, 7; Pyruvate Kinase, 8; Pyruvate Carboxylase, 9; Pyruvate Dehydrogenase.