Summary & Conclusion
Background

- The commonly practised pharmacological treatment of diabetes mellitus includes oral hypoglycemic agents and/or insulin injections. Insulin therapy offers effective glycemic control, but its shortcomings such as ineffectiveness on oral administration, short shelf-life, requirement of constant refrigeration and, in the event of excess dosage, fatal hypoglycemia limit its usage.

- Treatment with oral hypoglycemic agents such as sulfonylureas and biguanides is also limited due to their limited action, pharmacokinetic properties, secondary failure rates and the accompanying side effects.

- India is endowed with a rich tradition of herbal medicines as is evident from the fact that the *Susruta Samhita* differentiated between genetically and acquired forms of diabetes, and recommended many herbal medicines in different oral formulations for the treatment of disease. Even WHO has suggested investigating traditional methods of treatment for diabetes mellitus.

Hypothesis

The medicinal plants in the Indian systems of medicine are capable of not only controlling but also curing diabetes mellitus.

Plants chosen for the investigation

- 1) *Elephantopus scaber* Linn., commonly known as Anashovadhi, belonging to the Family Compositae. It is a mucilaginous plant, an astringent and used as tonic. A decoction of the root and the leaf is recommended for administration for dysuria, diarrhoea, dysentry and swelling or pain of the stomach.

- 2) *Eugenia jambolana* Lam., belonging to the Family Myrataceae. It is commonly called Jamun. Various medicinal properties of
E. *jambolana*, including its astringent, diuretic and anti-diabetic properties, have been described in traditional medicine.

- **3) Clitoria ternatea** Linn., belonging to the Family Fabaceae. It is a good "Medhya" (toning the brain or mental faculty) drug mainly used in the treatment of "Manasika roga" (mental illness), and is also said to be useful in hectic fever.

- **4) Phyllanthus emblica** Linn., belonging to the Family Euphorbiaceae. It is also known as amla or the Indian gooseberry, has been prescribed in Ayurveda, the ancient Indian systems of medicine, for treatment of several disorders such as common cold, scurvy, cancer and heart diseases.

- **5) Phyllanthus acidus** Linn., also belonging to the Family Euphorbiaceae. It is commonly called star gooseberry. It is less known in the Indian systems of medicine, but it is used in the folk medicine as remedy for ailments.

**Objectives**

- To find the maximally effective dose of aqueous and methanolic extracts of the chosen part(s) that would bring down the blood glucose level in diabetic rats.

- To find if oral administration of selected doses of the aqueous and methanolic extracts for several days would bring about any change in the biochemical parameters of diabetic rats, which are related to glucose homeostasis.

- To find the level of insulin in the serum of diabetic rats on treatment with the extracts, adopting RIA.

- To find the activities of glucokinase and glucose–6–phosphatase in the liver.
To find the toxic effects, if any, of the above mentioned plant parts in tissues like liver, kidney and pancreas.

To find the progressive and ultrastructural changes in the islets of Langerhans in response to the extract treatment.

To localize the presence of positively staining β-cells for insulin, i.e., insulin in the beta cells, adopting immunocytochemistry, so as to throw some light on the regeneration of beta cells in response to the treatment of the extracts.

To infer upon the hypoglycemic active principle(s) in the plant parts.

Methodologies

Biochemical parameters with respect to carbohydrate metabolism such as blood glucose, glycosylated hemoglobin, and liver and muscle glycogen were determined adopting appropriate methods.

The level of insulin in the serum was analysed adopting RIA.

The activities of the enzymes glucokinase and glucose-6-phosphatase of the serum were determined.

Biochemical parameters of the serum with respect to lipid metabolism, such as cholesterol, triglycerides and HDL-cholesterol, were also determined.

Biochemical parameters with respect to kidney function such as protein, urea and creatinine were also studied.

Light microscopic, TEM and immunocytochemical analyses of the islets of Langerhans were made.

The serum proteins were subjected to SDS-PAGE analysis.
Salient Features of the Results

1) Biochemical changes

- Alloxan-induced diabetes brought about increase in blood sugar and several corresponding changes in the biochemical parameters including severe hyperglycemia.
- Treatment of the plant extracts brought about considerable improvement in the situation.
- Glycosylated hemoglobin of the serum was brought down.
- Liver and muscle glycogen reserve increased.
- There was a marked increase in the level of insulin in serum.
- The activity of the enzyme glucokinase increased, whereas that of glucose-6-phosphatase decreased in the liver.
- Serum cholesterol and triglycerides decreased, whereas HDL-cholesterol increased.
- Serum protein registered an increase, whereas serum urea and creatinine decreased.
- Between the aqueous and methanolic extracts, the aqueous extract brought about a greater improvement than methanolic extract. Hence, aqueous extract-treated rats were subjected to histological, ultrastructural and electrophoretic studies.

2) Light microscopic changes in the islets of Langerhans

- The islets of Langerhans of control rats for diabetes had cells with round vesicular nuclei and pale pink cytoplasm.
- Most of the cells in the islets of Langerhans of diabetic rats had pycnotic nuclei; only a few cells were with normal architecture.
- The islets of the extract-treated diabetic rats were restored to normal histo-architecture.

3) Ultrastructural changes in the islets of Langerhans

- Based on the nature of the secretion granules in the cytoplasm \( \alpha- \), \( \beta- \) and \( \delta- \) cells were identified.
β-cells of control rats for diabetes contained numerous secretion granules in the cytoplasm, whereas in the β-cells of diabetic control rat, secretion granules were absent and, instead, there were numerous vacuoles.

β-cells of extract-treated diabetic rats contained secretion granules as well as vacuoles.

α- and δ-cells of untreated control, diabetic and extract-treated diabetic rats were normal.

4) Immunocytochemical study

The islets of Langerhans of control rats for diabetes contained insulin-positive β-cells in the islets of normal as well as extract-treated diabetic rats.

5) SDS – PAGE analysis

The protein profile of the serum of extract-treated diabetic rats showed improvement when compared to diabetic control rats.

The study leads to the following inferences

Aqueous and methanolic extracts of Elephantopus scaber (root and leaf), Eugenia jambolana (seed and bark), Clitoria ternatea (leaf and flower), Phyllanthus (emblica and acidus) fruit bring about recovery in alloxan-induced diabetic rats.

The extracts assume significance because of their ability to increase the insulin level of diabetic rats.

The observed decrease in glycosylated hemoglobin is due to increase in serum insulin level.

Increase in glycogen content after the extract treatment in insulin-dependent tissues like liver and skeletal muscle.
- Increase in the activity of the enzyme glucokinase and a decrease in the activity of glucose-6-phosphatase in the liver of extract-treated diabetic rats suggest that glycogenesis is enhanced and gluconeogenesis is inhibited.

- The plant extracts possess hypocholesterolemic and hypotriglyceridemic activities while at the same time increase HDL-cholesterol. This effect is due to an increase in insulin secretion that has led to decrease in fatty acid synthesis.

- Decrease in serum urea and creatinine suggests that the plant extracts are not toxic to the kidneys, but rectified the kidney impairment brought about by diabetes.

- Aqueous extracts of the plants are more effective than methanolic extracts in bringing about a hypoglycemic effect in alloxan-induced diabetic rats.

- Among the five plants chosen, aqueous extract of *Elephantopus scaber* (root/leaf) is more effective in bringing about hypoglycemic activity than the others. ESR treatment is more effective than ESL treatment in bringing about hypoglycemic effect.

- *Eugenia jambolana* treatment also has hypoglycemic activity, which is however greater than that of *Clitoria ternatea* extracts and less than that of *Elephantopus scaber* extracts. EJS extracts showed a greater hypoglycemic activity than EJB extracts.

- Between leaf and flower of *Clitoria ternatea*, CTL brings about greater hypoglycemic activity than CTF.

- *Phyllanthus* fruits (*emblica/acidus*) also has hypoglycemic activity in diabetic rats. PEF treatment is superior to PAF treatment.

- Aqueous extracts of the plants are more effective as hypoglycemic agents.
The biochemical mechanism of action of aqueous extracts of *Elephantopus scaber* (root/leaf), *Eugenia jambolana* (seed/bark), *Clitoria ternatea* (leaf/flower) and *Phyllanthus* fruits is brought about by regeneration of damaged β-cell as evident from histological, ultrastructural and immunocytochemical investigations.

In histological observations, islets of extract-treated diabetic rats are seen to have numerous cells with normal histoarchitecture.

Ultrastructural studies revealed β-cells of extract-treated diabetic rats to contain granulated secretory vesicles.

There were numerous cells positively stained for insulin i.e., β-cells, in immunocytochemically stained islets of extract-treated diabetic rats.

The protein profile of the serum of extract-treated diabetic rats is similar to that of untreated rat serum.

The active principles in the above mentioned plants are flavonoids, glycosides and others which may act synergistically to regenerate the damaged endocrine pancreas and thereby stimulation of insulin secretion in β-cells as revealed by insulin assay, LM, TEM and immunocytochemical staining.