CHAPTER – 3

Aim & Objective
3. AIM AND OBJECTIVE OF STUDY

Medicinal plants are still an important source of novel pharmaceutical products and natural products still remain as one of the best reservoir of novel structural-typed bioactive compounds. More than 25% of present medicine comes from natural products and an additional 25% are structural alteration of the lead compounds from natural source. It is estimated that only 15% of higher plants have been investigated for potentially useful biological activity. In spite of the presence of known antidiabetic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease. Numerous traditional plant treatments for diabetes are used throughout the world. Plant drugs and herbal formulations are frequently considered to be less toxic and free from side effects than synthetic one (Craker et al., 2006).

Based on the WHO commendation, hypoglycemic agents of plant origin, used in traditional medicine are significant. The antihyperglycemic properties of these plants are due to their capability to reinstate the function of pancreatic tissues by causing a raise in insulin output or decline in the intestinal absorption of glucose. Therefore treatment with herbal drugs has an effect on protecting β cells and smoothing out fluctuation in glucose levels. In general, there is very little biological knowledge on the specific modes of action in the treatment of diabetes, but most of the plants have been found to contain substances like phenolics, glycosides, alkaloids, terpenoids, flavonoids etc., that are normally concerned as having antidiabetic effects.

Hence, in modern days, huge attention has been directed towards recognition of plants with antidiabetic ability that may be used effectively for human consumption (Elder, 2004). There has been rapid development of different classes of antihyperglycemic drugs with distinctive pharmacological mechanism of action and, also, they have various toxicological profiles. Numbers of medications are reported for the management of hyperglycemia like insulin, sulphonylureas, biguanides, thiazolidinedione, alpha-glucosidase inhibitors, glucagon-like peptide-1 analogues, glycosourics and dipeptidyl peptidase- IV inhibitors. Side effect of antihyperglycemic drugs may cause agitation, altered behaviour, excess sweating, inaudible speech, tachycardia, seizures, and coma. Side effect of subcutaneous dose of insulin is related with deep risk blurred vision and hypoglycemia. Sulfonylureas can cause hypoglycemia, which stimulates appetite and leads to weight gain. Biguanides causes anorexia and encourage weight loss. Thiazolidinediones like pioglitazone, rosiglitazone can cause hepatic dysfunctioning as an adverse effect on regular treatment. Still there is a challenge to the medical system for Management of diabetes without any side effects. However, natural remedies are widely used.
around the world to treat diabetes, but medical research does not support their effectiveness (Liu et al., 2004). So, the search for natural drugs from medicinal plants is being increased because of its fewer side effects, willingly availability and low cost. Thus the scientific validation of medicinal plants traditionally used in the treatment and management of diabetes is demanded.

The literature review revealed that antidiabetic activity of aerial parts of *A. mexicana* and *H. suaveolens* has not been experimentally studied, as well as no detailed study has been carried out on the modulation of oxidative stress, effect on lipid profile and hepatic enzymes of glucose metabolism associated with DM in experimental induced diabetic rats. From the literature survey it has been found that the two selected plants are being used traditionally in the treatment of diabetes, based upon their traditional and ethnopharmacological information’s. However, a lot of research work in various areas has been reported on *A. mexicana* and *H. suaveolens*.

Therefore, based on the exhaustive literature survey, the objective of the present work focused on the search of herb, which will found more suitable among the reported herbs/ herbal preparations, towards the control of blood glucose level in hyperglycemic condition based on their traditional claim of the selected herbs. In the present investigation two herbs; *Argemone mexicana* L and *Hyptis suaveolens* L were considered based on their traditional claim and related literature information.

The objective of the present study will be carried out by the following sequence based on bioassay guides principles.

- Blood glucose lowering effect of the plant extract and fractions in both acute and sub-acute *in-vivo* antidiabetic models.
- Evaluation of Biochemical parameters in support of the antidiabetic activities.
- Study of *In-vitro* and *in-vivo* antioxidant activity of extracts and fractions.
- Toxicity and Safety profile study of the plant extracts and fractions.
- Isolation, characterization and *in silico* study of phytoconstituents from the potent bioactive fraction.
3.1. PLAN OF WORK

The present work was designed by the investigators, bases on different approaches of bioassay guided study, which will scientifically validate the traditional claim of the selected plants in an empirical way.

- Selection of plants based upon literature survey and traditional claim
- Identification, authentication of Plant(s) and collection of plant materials
- Preparation of extracts and fractions
- Preliminary phytochemical study
- Studies of the extracts followed by suitable fractions in support of the objectives.

Anti-diabetic study of extracts

- Acute toxicity studies of different extracts
- Effect of the extracts on normoglycemic rats
- Oral Glucose Tolerance Test of the extracts
- Effect on blood glucose level of the extracts in alloxan induce hyper glycemic rats (Acute and sub-acute models)
- Effect of the extracts on serum biochemical parameters
- Effect on change of body weight in sub-acute model

Anti-diabetic study of fractions

- Solvent-solvent fractionation of best extract
- Acute toxicity studies of different fractions
- Effect of the different fractions on normoglycemic rats
- Sub acute antidiabetic study of the fractions
- Effect of the fractions on serum biochemical parameters
- Effect of the fractions on serum lipid profile
- Effect on body weight
- Effect on food and water intake
- In vitro alpha amylase inhibitory effect of bioactive fractions
Safety profile study

Liver toxicity study by measurement of level of different liver enzymes

Anti-oxidant activity study

\textit{In vitro} antioxidant activity of different extracts

- Reduction of 1, 1-diphenyl- 2- picryl hydrazyl (DPPH)
- Hydrogen peroxide ($H_2O_2$) radical scavenging

\textit{In vitro} antioxidant activity of potent fraction

- Reduction of 1, 1-diphenyl- 2- picryl hydrazyl (DPPH)
- Nitric oxide scavenging activity
- ABTS scavenging activity
- Super oxide radical Scavenging

\textit{In vivo} antioxidant activity of fractions

By determination of level of antioxidants enzymes in pancreas, liver and kidney

- Superoxide dismutase (SOD)
- Catalase (CAT)
- Reduced Glutathione
- Glutathione-S-transferase (GST)
- Total Protein level

Isolation, characterization and \textit{in-silico} studies

- Isolation of phytochemical in the potent fraction by column chromatographic techniques
- Characterization of isolated compound by spectral analysis using spectrophotometers
- To identify the target protein sites for interaction of isolated phytochemical by docking method using \textit{in-silico} techniques.