CHAPTER – V

DISCUSSION

The synthetic drugs used for the treatment of diabetes produce lot of adverse effects. The drugs of natural origin have stood the test of time for their safety, efficiency, cultural acceptability and lesser side effects. Hence it has been planned to study scientifically and systematically some traditional herbal formulations, used in the treatment of diabetes and to come out with an herbal remedy that would benefit the people with diabetes.

Based on an ethnomedical survey and an indepth, literature survey two reputed Indian System of Medicine formulations were selected for the study. They are athi uchidum and Nagaparpam coded as FS002 and NP003 respectively. For preparation of these herbal formulations, the herbal ingredients should be pure, authenticated and standardised. The raw materials used for making the formulations were collected, identified and authenticated. The herbal raw materials of the selected formulations were subjected to organoleptic, microscopical and physical studies.

Pharmacognostical anatomy

Plant anatomical studies play a crucial role in plant identification and standardisation. The term bark refers to all the tissues outside the vascular cambium of the stem and root. The tissues of the bark include periderm and
secondary phloem. Periderm is also known as outer bark and the secondary phloem is known as the inner bark (Trockenbrodt 1990). Periderm is produced by a lateral meristem called phellogen and the secondary phloem is produced by the vascular cambium. So, the periderm and secondary phloem are different in their development. Periderm consists of an outer, dead, suberised tissue called phellem and inner living tissue called phelloderm. Phellem is productive in function. The phelloderm is storage in function. The phelloderm may be broad of it may be a single zone or several zones alternating with non peridermousn tissues such as dead cortical tissues or secondary phloem. Such compound structure is called rhytidome.

The periderm may originate superficially from epidermis or subepidermal layers; it may also originate from deeper layers of the cortex or from the secondary phloem. The surface of the bark will exhibit several features such as colour, texture, fissuring and scaling. All these features are of diagnostic interest (Fig.1.1).

The inner bark usually consists of outer collapsed phloem and inner noncollapsed phloem.

Phloem: The collapsed phloem is the store house of many ergastic compounds such as starch grains, crystals, tannins and lipids. The types of storage products and their distribution patterns are valuable observations in the study of the bark.

The phloem rays play significant role in determining the surface features as well as internal structures of the bark. Sieve plates, sieve tube members,
companion cells, and axial phloem parenchyma are the components of diagnostic potentials of the bark.

Plant barks are so much of importance in the medicinal and industrial arena, that their study gains significance in pharmacognosy, phytochemistry and pharmacological studies.

The bark of *Ficus racemosa Linn* were subjected to microscopical studies.

The bark of *Ficus racemosa Linn* could be divided into (a) outer rhytidome (b) inner secondary phloem or inner bark.

The unique feature of the outer bark is the presence of a structure ‘periderm tubes’. The periderm tubes consists of an irregular shape of circular, cuboid layers of phellem.

The inner secondary phloem is a major part of the bark. The inner bark consists of secondary phloem elements. The secondary phloem can be divided into two zones, namely outer collapsed phloem and inner non collapsed phloem.

When the secondary phloem is viewed under polarised light microscope, the collapsed phloem cells contain large calcium oxalate crystals and dense starch grains. The crystals are of pneumatic type whereas the starch grains are small, circular and concentric.

The presence of periderm tubes in the outer bark, secondary phloem in the inner bark which is subdivided into collapsed and non-collapsed phloem,
pneumatic crystals of calcium oxalate, concentric starch grains, phloem fibres
and geodiametric stone cells are observed in, the cross sections of the bark and
its powder microscopy are of diagnostic importance.

**Pharmacognostical studies**

Medicinal plant materials should be entirely free from visible sign of
contamination, by moulds or insects and other animal contamination, including
animal excreta. No obscured odour, discolouration, slime or signs of
deterioration should be detected. The herbal ingredients should be stored in a
particular environment to avoid formation of moulds, since they produce
aflatoxins.

Any soil, stones, sand, dust and other foreign organic matter must be
removed before medicinal plant materials are cut or ground for testing.

Macroscopic examination was employed for determination of foreign
matter (WHO Guidelines, 2002).

When analysed by the macroscopic examination the foreign matter
present in both the formulations were found to be within normal limits and
hence they could be used for animal and human studies.

**Physical evaluation**

In order to standardize the formulations and to determine the quality and
purity of the formulations the total ash, acid insoluble ash and water soluble
ash were determined in three different sample formulations. The total ash
includes both physiological ash, which is derived from the plant itself and non-physiological ash which is the residue of extraneous matter adhering to the plant surface e.g. (sand and soil).

The low acid insoluble and water soluble ash values of formulation FS002 indicates that extraneous matter were kept to minimum in the preparation of these formulations.

The extracts obtained by exhausting crude drugs are indicative of approximate measure of their chemical constituents. Taking into consideration the diversity in chemical nature and properties of constituents of the drugs, various solvents were used for determination of extractives.

The method determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material. The methanolic \((18.76 \pm 0.32)\) and alcoholic \((16.7 \pm 0.32)\) extractive values were the maximum values for formulation FS002 whereas the herbo mineral preparation NP003 showed maximum extractive values for water \((10.5 \pm 0.22)\) alcohol \((14.5\pm 0.22)\) and methanol \((16.426 \pm 0.46)\). These values showed that the active constituents in these formulation were extracted maximum in the above mentioned solvents.

An excess of water in medicinal plant materials will encourage microbial growth, the growth of fungi or insects and deterioration following hydrolysis. The limits of water content should therefore be set for every given plant material. Loss on drying was determined by gravimetric method, showed that the formulation FS002 showed \(2.578 \pm 0.32\%\) and \(6.578 \pm 0.45\%\) before
and after steaming respectively and for NP003 it was 1.376 ± 0.25%. The values of the moisture content were within normal limits prescribed for herbal materials (WHO guidelines 2002).

The particle size of the formulations, assessed by linear microscopy method was found to be between 25 to 30 μ, which was reported to be ideal for oral absorption of these formulations.

As a part of the standardization of herbo-mineral formulation NP003, the amount of zinc was estimated using Atomic Absorption Spectroscopy. The amount of zinc was found to be 82.06 ± 2.50% w/w.

**Phytochemical study**

Bark is one of important parts of the plant in storing many valuable medicinal compounds. Wax, fat, fatty acids, volatile oils, resins, alcohols and hydrocarbons have been extracted from the bark. Aqueous extracts are rich in tannins, soluble carbohydrates, mucilage, gum, pectin and glycosides. Alkaline extracts yield high molecular weight phenolic acids. Phlobaphenes and alkaloids have been identified in the ethanol fractions of the bark.

Qualitative chemical test or phytochemical screening of the bark of *Ficus racemosa* Linn revealed the presence of sterols, tanins, saponin and reducing sugars. Whereas its formulation showed the presence of similar phytochemicals, but in addition to that showed the presence of flavonoids (Table – 7). Phytoconstituents identified by phytochemical screening were of similar nature, when compared to the compounds reported to be isolated from
these plants.
Testing for microbial contamination

Medicinal plant materials normally carry a great number of bacteria and moulds, often originating in soil. While a large range of bacteria and fungi form the naturally occurring microflora of herbs, aerobic spore forming bacteria would be also present. The determination of *Escheria coli* and moulds indicate the quality of production. Both the formulation FS002 and NP003 passes the test of microbial standardisation, as they did not show any visible growth of bacteria or contamination. The total microbial count were within limits for both the formulation FS002 and NP003. The formulation FS002, stored after 90 days showed excessive limits of total viable count >10^4, which infer showed that it was unfit for human consumption after 90 days.

Instrumental analysis

HPTLC studies and finger printing is fast emerging as one of the major tools by which quality of herbs and its formulations are assessed. It is also helpful in identification of various chemical markers of the herbs (Sane et al., 1998).

To minimise batch to batch variations and to add scientific validity to herbal formulations, it is necessary that like modern drugs, herbal drugs should also be analysed and proper quality control techniques should be developed to verify the quality and quantity of the herbs added in the formulation.

The study would be also useful to standardise the bark of *Ficus racemosa* and elucidate the HPTLC finger printing (Sarawathy, 2003) of the bark, which shows 4, 8 and 4 peaks when scanned at 254, 366 and 600 nm
The bark of *Ficus racemosa* (FR sample) and the formulation FS002 showed a superimposition and a similarity of 8 peaks corresponding to similar \( R_f \) values were obtained, when densitometric scanning was done at different wavelengths (254, 366 and 660 nm) indicating that the active constituents remain intact even after processing the bark into a formulation.

**Preliminary pharmacological studies**

The hypoglycemic effect of the siddha formulations FS002 and NP003 on fasting blood glucose level of rabbits were studied and it was found that the fasting blood glucose level was decreased to a maximum of 20 mg/dL and 25 mg/dL as compared to the baseline values. Statistical analysis revealed a significant hypoglycemic (\( P<0.05, P<0.01 \)) activity as compared to the vehicle group.

The formulation were subjected to screening for antihyperglycemic activity by OGTT model in albino rats. The glucose levels after glucose challenge of 1 g/kg body weight raised to a level of nearly 50 mg/dL. The elevated blood glucose levels were suppressed by both the formulations to an extent of 45 mg/dL and 55 mg/dL for the formulation FS002 and NP003 respectively. Both the formulations possessed statistically significant anti-hyperglycemic (\( P<0.02, P<0.001 \)) activity as compared to control group.

**Advanced pharmacological studies**

Streptozotocin is probably the most widely used agents producing
IDDM and NIDDM in experimental animals. These animals also exhibit diabetic complications such as myocardial (Abede and Mchood, 1991), Cardiovascular (Ozeelikay, et al., 1994), gastrointestinal (Ozlunk et al., 1992), tracheal (Ozansoy et al., 1993), kidney (Reddi et al., 1991), Urinary bladder (Jeremy et al., 1993) and connective tissue dysfunctions similar to diabetic patients. Streptozotocin induced NIDDM in rats constitute an invaluable pharmacological tool for screening for antidiabetic activity because most of the diabetic patients are non-insulin dependent in nature.

So the formulations NP003 and FS002 were studied for anti-diabetic activity in streptozotocin induced diabetic model in adult wistar albino rats. The alcoholic extract, formulations FS002 and NP003 exhibited statistically significant antidiabetic activity in streptozotocin induced diabetic rats as evident from serum glucose and hepatic glycogen levels.

The effect of the extract, Formulations FS002 and NP003 on body weight, liver glycogen content and serum glucose were tabulated (Table – 16, 17). A marginal reduction in the body weight of the animals in these groups was observed, as compared to initial body weights, but fell short of statistical significance. There was marked reduction in liver glycogen level (in 15 days) in streptozotocin diabetic animals. The herbal extract, FS002 treatment remarkably attenuated this reduction in glycogen content, but NP003 showed a much less increase in glycogen content.

So the herbal extract (FG) and its formulation FS002 would have stimulated glycogenesis and or inhibited glycogenolysis in diabetic rat liver, whereas the formulation NP003 could have acted by some other mechanisms
i.e. stimulating insulin release etc. Further the formulation NP003 contains, zinc which have been reported in literature to release insulin from β-cells.

The elevated serum glucose levels were significantly decreased by the herbal extract (FG) and its formulation, FS002 and NP003 and the effects were comparable to that of the standard drug, Glibenclamide.

**Toxicological studies**

FS002 and NP003, polyherbal formulations intended to be used in diabetic patients has been screened for toxic effects. The formulation were subjected to acute and sub acute toxicity studies.

The results shows that very high doses were tolerated by the mice without producing any toxicity symptoms. The ingredients present in the formulation FS002 were phytochemical in origin and contains sterols, triterpenoid saponins, aminoacids and flavonoids, which was confirmed by subjecting the formulation and its ingredients to phytochemical screening, HPTLC studies and comparing the data with reported literature. Since a number of phytoconstituents are present in the formulation, these experiments were designed to screen for any toxic effects. Since there are no toxic effects observed it could be inferred that the basic principle in the use of crude plant products or polyherbal preparations in traditional medicine was that the toxic effect of one component is nullified by the protective effect of the other components, without interfering with their therapeutic properties.

The finding that daily administration of FS002 at different doses of 0.2, 0.4, 1 and 2g/kg body weight and NP003 of 20, 40, 80, 200 mg/kg body weight
were well tolerated and there was no cumulative toxicity as evidenced by biochemical and histopathological data, shows that these drugs could be used effectively in the treatment of chronic diseases.

**Antioxidant activity**

Free radicals have implicated in a variety of conditions including inflammation, atherosclerosis, diabetes, ageing and hepatic toxicities (Halbiwell et al., 1999). Free radicals attack membrane lipids thereby generating lipid radicals and these lipid radicals can combine with oxygen producing peroxyl radicals which is responsible for cellular injury in different pathological process.

Antioxidants are compounds which act as inhibitors of the oxidative process. They are quite large in number and diverse in nature, which oppose the process of oxidation largely by neutralizing free radicals and inhibit oxidative chain reaction. Since the formulation FS002 possess phytochemicals, with reported antioxidant activity, the formulation was screened for antioxidant activity in different invitro models. The antioxidant effect of the bark and its formulation FS002 were studied using DPPH and in-vitro lipid peroxidation techniques. The ethanolic extract of the bark of *Ficus racemosa* and similar extract of the formulation showed significant free radical scavenging activity in these systems. These IC$_{50}$ values of free radical scavenging activity of ethanolic extract of bark (EE) and its formulation FS002 (EE1) was found to be 74.5 and 54.5 µg/mL in DPPH system. Thus it was found that the formulation was more potent than its ingredient, the bark of *Ficus racemosa*. 
The IC$_{50}$ values for *in-vitro* lipid peroxidation activity as evidenced by decrease in malionaldehyde production was found to be 53.6 and 66.4 μg/mL for EE and EE1 respectively which shows that the ethanolic extract of bark was found to be more potent in lipid peroxidation model.

Plant derivatives with purported hypoglycemic properties have been used in folk medicine and traditional healing systems around the world (e.g. Native American, Indian, Jewish, (Yaniv et al., 1987), Chinese (Covington MB, 2001), East Indian, Mexican). Many Modern pharmaceuticals used in conventional medicine today also have natural plant origins, like the claimed example of Metformin from flowering plant, *Galgena officinalis* (Goat's Rue or French Lilac), which was a common traditional remedy for diabetes (Pandey et al., 1995; Oubic et al., 1997). Similarly the use of vitamin and mineral supplements for primary or secondary disease prevention is of increasing interest (O'Connell, B., 2001).

Studies regarding the efficacy and safety of herb or other dietary supplements are scarces and unreported. Hence the present study has been conducted to evaluate the herbal and herbo-mineral formulations in the treatment of diabetes mellitus. Though various antidiabetic drugs are available the problem of achieving a successful glycemic control and prevention of complications continue to persist. In this context indigenous drugs have proved their worth (Prasanna Kumar et al., 2003). Both the formulation FS002 and NP003 were subjected to clinical studies after a detailed pharmacological and toxicological studies. Both the formulations showed good plasma glucose lowering effects. The formulation FS002 decreased, the fasting and postprandial blood glucose level to an extent of 25 mg/dL and 40 mg/dL.
respectively, (Table – 26) whereas NP003 decreased to an extent of 39 mg/dL and 70 mg/dL respectively, when assessed for a period of 3 months (Table 27). These drugs may stimulate β-cells of langerhans or it may increase the peripheral utilization of glucose, but their exact mechanisms of action needs further investigation.

The HbA₁c levels reflects the glycemic control over the previous 2 to 3 months. In the present study with FS002 and NP003, one unit decrease in the HbA₁C values produced a decrease of 50 mg/dL (33%) of the blood glucose level (Table – 26, 27).

Both the formulations significantly decreased the blood urea and serum creatinine in patients with elevated level. These show that the formulations could be additionally beneficial in bring down the hepatic and renal complications due to diabetes. The data also shows that these formulations were safe to be administered to patients with hepatic and nephrotic injury due to diabetes.