CHAPTER – VI

SUMMARY AND CONCLUSION

Two reputed siddha herbal antidiabetic formulations (herbal and herbomineral) were selected, based on a ethnomedical survey for a detailed pharmacognostical, Pharmacological, toxicological and clinical investigation.

1. The selected formulation were prepared as per the procedure laid down in the official text and subjected to preliminary pharmacological screening for hypoglycemic activity using fasting albino rabbits. The formulations FS002 and NP003 reduced the blood glucose level significantly. The herbomineral NP003, was found to be more potent than FS002 in reduciang the blood glucose level.

2. Since the formulation showed significant pharmacological activity and as a part of the Pharmacognostical studies of the selected formulations, FS002 and NP003, the ingredients of the formulation were selected, identified, pharmacognostically studied and processed into a formulation. The herbal raw materials of the selected formulation FS002 were Bark of *Ficus racemosa* and Sesamum Indicum cake. The bark of *Ficus racemosa* Linn were anatomically studied and was reported for
the first time. A detailed anatomical description of the anatomical structures of the bark of *Ficus racemosa* would be guidelines for researchers who wish to study the bark of others species of *Ficus*. The bark shows unique periderm layers called rhytidome, presence of periderm tubes, obliterated cortex, in the outer part. The inner bark which is the major part shows the presence of secondary phloem elements, such as collapsed phloem and non-collapsed phloem.

The collapsed phloem contains collapsed severe elements companion cells, secondary phloem rays and axial parenchyma. The non collapsed phloem shows the presence of sieve tube, sieve plate, companion cells, large prismatic calcium oxalate crystals and dense starch grains.

The powder microscopy of the bark shows prismatic calcium oxalate crystals, parenchyma cells, starch grains, phloem fibres and isodiametric or elongated stone cells. The organoleptic characters of marc of *Sessamum indicum* seeds were studied and documented.

3. Macroscopic examination of the formulations was done to detect the presence of foreign matter according to WHO guidelines. The foreign matter present in the formulation were found to be within normal limits. The moisture content in these formulations were determined by Gravimetric method according to WHO guidelines. Both the formulations when assessed showed the moisture content within normal limits, since excess of moisture
would encourage microbial growth and deterioration by hydrolysis.

4. In order to standardize the formulation and to determine the quality and purity of the formulation the Total ash, acid insoluble ash and water soluble ash were determined in the three different batches of the formulation.

5. The higher acid insoluble ash value of NP003 (10.59 ± 0.54) may be because of the presence of large amount of silica. Since the preparation was itself a bhasma and involves the use of mud vessels for its preparation, its high value was expected. The low acid insoluble ash value (1.39 ± 0.14) of FS002 indicates that extraneous matter were kept to minimum in the preparation of the formulation.

6. As a part of the pharmacognostical standardization the extractive values of the formulation in different solvents were determined as exact chemical moieties which were present in the formulation were not known. The extracts obtained by exhausting crude drugs are indicative of approximate measure of their chemical constituents.

The methanolic and alcoholic extractive values (18.76 ± 0.32; 14.5 ± 0.22) were maximum for formulation FS002 where as the herbo-mineral preparation NP003 showed a value of 14.5 ± 0.22, 10.5 ± 0.22, 16.426 ± 0.46 for alcohol, water and methanol respectively as maximum, which shows the
chemical constituents extractable to the maximum in these solvents and corresponding extracts could be used for confirming the biological activity of the formulations.

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7. As a part of the standardization of the formulation FS002 and NP003, the particle size was determined using linear microscopy method. The particle size of FS002 and NP003 were found to be 29.5 ± 10.5 and 25.5 ± 9.65 respectively.

8. The herbomineral formulation NP003, contains Zinc as the main ingredient. So in order to standardize NP003, the amount of Zinc present was estimated using Atomic absorption spectroscopy. The amount of Zinc was found to be 82.06 ± 2.50% W/W.

9. The *Ficus racemosa Linn* bark and its formulation FS002 were subjected to phytochemical screening to find the chemical constituents present. The bark revealed the presence of sterols, tannin, saponins and reducing sugars, where as its formulation showed the presence of similar constituents, but in addition to that showed the presence of flavonoids. The phytochemical constituents identified correlated with the literature reported for the herbal raw materials.
10. Microbial evaluation of the formulations were performed using standard guidelines. The determination of Enterobacteria, presence of E.coli and total viable aerobic count were assessed in these formulations. Both the formulations FS002 and NP003 passed the test of microbial standardisation, since no visible growth of the bacteria or contamination was observed. The total microbial count were within limits for both the formulation FS002 and NP003. The formulation FS002 showed excessive limits of total viable count > 10^4 after 90 days of storage, which showed that it was unfit for human consumption after 90 days.

11. The bark and its formulation FS002 were subjected to HPTCLC analysis. HPTLC finger printing analysis of the bark of Ficus racemosa Linn were reported for the first time. The bark shows 4, 8 and 4 (total 12 peaks) when scanned at different wavelengths at 254; 366 and 600 nm respectively. The data would be useful for the standardization of the bark. The denistometric scanning of the formulation revealed 3,6,8 peaks at different wavelengths of 254, 366 and 600 nm respectively, which could be used as HPTLC finger print of the formulation FS002. The R_f values of the corresponding peaks were calculated and tabulated. The 8 peaks for the bark and its formulation showed a superimposition and similar R_f values which indicates that active constituents remain intact even after processing the bark into a formulation.

12. The formulations were subjected to screening for antihyperglycemic activity by oral glucose tolerance test model
(glucose loaded rats). Both the formulation FS002 and NP003 showed significant antihyperglycemic activity as compared to the control group. The herbo-mineral formulation NP003 was found to be more potent than FS002.

13. The formulations FS002 and NP003 were also studied for antidiabetic activity with Streptozotocin induced diabetic model which mimicks NIDDM diabetes in experimental animals. The effect of the ethanolic extract of the formulations FS002 and NP003 on body weight, liver glycogen content and serum glucose were studied.

The formulations showed significant antidiabetic activity and the elevated serum glucose levels were reduced to almost normal levels by the herbal extract and its formulations FS002 and NP003 and the effects were comparable to that of the standard drug, glibenclamide. Both the formulation showed no significant reduction in body weights and the herbomineral preparation NP003 was less increasing in reducing the liver glycogen content as compared to the formulation FS002.

14. The two polyherbal formulation FS002 and NP003, were screened for toxic effects. FS002 and NP003 were administered orally in graded does of 0.2 to 4 g/kg land 20 to 2000 mg/kg body weight respectively to albino mice for acute toxicity studies and albino rats for sub-acute toxicity studies. No acute mortality were observed for the formulations even at the highest dose of 2 g/kg body weight and 200 mg/kg body weight for FS002 and
NP003 respectively. In sub-acute toxicity studies the animals showed no signs of toxicity produced by these drugs. The hematological observations showed no significant difference in RBC count, hemoglobin, serum alkaline phosphatase, acid phosphatase, ALT, AST, urea and creatinine levels as compared to control group.

Histopathological examination of the organs did not reveal any pathological changes for both the formulations. These observations were similar in male and female rats. These results show that a very high doses of these drugs have been tolerated by albino mice and rats.

15. 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 ethanolic extract of the bark and its formulation were studied using DPPH and invitro lipid peroxidation techniques. The ethanolic extract of the bark and similar extract of the formulation showed significant free radical scavenging activity in these systems. The IC\textsubscript{50} values for the free radical scavenging activity of the ethanolic extract of the bark (EE) and its formulation (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 the bark.
The IC	extsubscript{50} valve for inhibition of \textit{invitro} lipid peroxidation activity was found to be 53.6 and 66.4 μg/mL for EE and EE1 respectively.

16. Both the formulations FS002 and NP003 were subjected to clinical evaluation in type 2 diabetes patients after a detailed pharmacognostical, pharmacological and toxicological studies. Both the formulations showed significant plasma glucose lowering effects and reduction in HbA	extsubscript{1c} levels.

Both the formulations significantly decreased the blood urea and serum creatinine in patients with elevated levels. Thus these formulations could be additionally beneficial in reducing the hepatic and renal complications due to diabetes.

Studies lead to the conclusion that herbal (FS002) and herbo-mineral formulations (NP003) could be used for the treatment of diabetes mellitus, as they are found to be potent and safe in both pre-clinical and clinical studies. However elucidation of exact mechanism of action of benefical effects of these formulations need further investigation. More randomized controlled trials in large patient populations has to be carried out before determining the status of these drugs in the therapy of diabetes.